

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

AO

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07F 9/30, A61K 31/66, C07F 9/58, 9/32, 9/40, 9/44		A1	(11) International Publication Number: WO 99/33847
			(43) International Publication Date: 8 July 1999 (08.07.99)
(21) International Application Number: PCT/US98/27175		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 21 December 1998 (21.12.98)			
(30) Priority Data: 09/002,147 31 December 1997 (31.12.97) US			
(71) Applicant: GUILFORD PHARMACEUTICALS INC. [US/US]; 6611 Tributary Street, Baltimore, MD 21224 (US).			
(72) Inventors: JACKSON, Paul, F.; 102 Glenmore Court, Bel Air, MD 21014 (US). LI, Weixing; Apartment E, 40 Leatherwood Place, Baltimore, MD 21237 (US). MACLIN, Keith, M.; 5005 Remmel Avenue, Baltimore, MD 21206 (US). TAYS, Kevin, L.; Apartment 1415, 6947 Old Waterloo Road, Elkridge, MD 21227 (US). TSUKAMOTO, Takashi; 4J Cameron Court, Baltimore, MD 21236 (US). SLUSHER, Barbara, S.; 7424 Longfield Drive, Kingsville, MD 21087 (US).		Published <i>With international search report.</i> <i>With amended claims.</i>	
(74) Agent: NATH, Gary, M.; Nath & Associates, 6th floor, 1030 15th Street, Washington, DC 20005 (US).			

(54) Title: PHOSPHINIC ALKANOIC ACID DERIVATIVES

(57) Abstract

The present invention relates to phosphinic alkanolic acid derivatives that inhibit N-Acetylated α -Linked Acidic Dipeptidase (NAALDase) enzyme activity, pharmaceutical compositions comprising the same, and methods of using the same of inhibit NAALDase activity and to treat glutamate abnormalities and prostate diseases.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PHOSPHINIC ALKANOIC ACID DERIVATIVES

This application is a continuation-in-part (CIP) of U.S. Patent Application No. 08/665,775 (Attorney Docket No. 23060), filed June 17, 1996, Slusher et al., entitled "Methods of Cancer Treatment Using NAALADase Inhibitors"; U.S. Patent Application No. 08/864,545 (Attorney Docket No. 23060-X), filed May 28, 1997, Slusher et al., entitled "Methods of Cancer Treatment Using NAALADase Inhibitors"; U.S. Patent Application No. 08/718,703 (Attorney Docket No. 23125), filed September 27, 1996, Slusher et al., entitled "Treatment of Global and Focal Ischemia Using NAALADase Inhibitors"; U.S. Patent Application No. 08/884,479 (Attorney Docket No. 23125-X), filed June 27, 1997, Slusher et al., entitled "Pharmaceutical Compositions and Methods of Treating a Glutamate Abnormality and Effecting a Neuronal Activity in an Animal Using NAALADase Inhibitors"; U.S. Patent Application No. 08/899,319 (Attorney Docket No. 23125-XX), filed July 23, 1997, Slusher et al., entitled "Pharmaceutical Compositions and Methods of Treating Compulsive Disorders Using NAALADase Inhibitors"; and U.S. Patent Application No. 08/900,194 (Attorney Docket No. 23060-XX), filed July 29, 1997, Jackson et al., entitled "Inhibitors of NAALADase Enzyme Activity".

BACKGROUND OF THE INVENTION

1. Field of the Invention

5 The present invention relate to phosphinic alkanolic acid derivatives that inhibit N-Acetylated α -Linked Acidic Dipeptidase (NAALADase) enzyme activity, pharmaceutical compositions comprising the same, and methods of using the same to inhibit NAALADase activity and to treat glutamate abnormalities and prostate diseases.

2. Description of the Prior Art

Glutamate Abnormalities

15 Glutamate serves as the predominant excitatory neurotransmitter in the central nervous system (CNS). Neurons release glutamate in great quantities when they are deprived of oxygen, as may occur during an ischemic brain insult such as a stroke or a heart attack. This excess release of glutamate in turn causes over-stimulation (excitotoxicity) of N-methyl-D-aspartate (NMDA), AMPA, Kainate and MGR receptors. When glutamate binds to these receptors, ion channels in the receptors open, permitting flows of ions across their cell membranes, e.g., Ca^{2+} and Na^{+} into the cells and K^{+} out of the cells. These flows of ions, especially the influx of

Ca²⁺, cause over-stimulation of the neurons. The over-stimulated neurons secrete more glutamate, creating a domino-effect which ultimately results in cell death via the production of proteases, lipases and free radicals.

5 Excessive activation of glutamate receptors has been implicated in various neurological diseases and conditions, including epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease, schizophrenia,
10 chronic pain, ischemia and neuronal loss following hypoxia, hypoglycemia, ischemia, trauma, and nervous insult. Recent studies have also advanced a glutamatergic basis for compulsive disorders, particularly drug dependence.

15 As an example, neurophysiological and pathological effects of ethanol have been found to be mediated through the glutamatergic system. Specifically, acute exposure to ethanol disrupts glutamatergic neurotransmission by inhibiting ion flow through channels in glutamate
20 receptors, whereas chronic exposure up-regulates the number of glutamate receptors and thereby increases ion flow. Acute withdrawal from ethanol results in hyperexcitability and seizures in the presence of up-regulated channels, thereby making postsynaptic neurons
25 vulnerable to excitotoxic damage.

Post mortem examinations of histologically normal

brains from alcoholics have shown that chronic alcoholism moderately increases the density of the NMDA subtype of glutamate receptors in the frontal cortex. This up-regulation may represent a stage of ethanol-induced chronic neurotoxicity. As such, neurobiological effects of alcoholism, including intoxication, withdrawal seizures, delirium tremens, Wernicke-Korsakoff syndrome and fetal alcohol syndrome, can be understood as a spectrum of the consequences of ethanol's effect on the glutamatergic system. In this regard, alcoholism may be considered another member of the expanding family of glutamate-related neurological disorders.

The glutamatergic system has also been implicated in the behavioral effects of other abused drugs. For example, studies have shown that glutamatergic antagonists block motor-stimulating activities induced by amphetamine and cocaine, and glutamatergic agonists cause the same stereotypy as that produced by amphetamine. These results represent pharmacological evidence that the expression of the stereotypic effect of psychomotor stimulants involves the glutamatergic system.

Epidemiologic studies have revealed a strong correlation between drug dependence and other compulsive disorders. Additionally, a common genetic anomaly has been found among people with alcoholism, cocaine dependence, nicotine dependence, pathological gambling,

attention deficit disorder (ADD), Tourette's syndrome, compulsive overeating and obesity. Such disorders are believed to be manifestations of the effects of excitotoxicity.

5 Attempts to prevent excitotoxicity by blocking NMDA, AMPA, Kainate and MGR receptors have proven difficult because each receptor has multiple sites to which glutamate may bind. Many of the compositions that are effective in blocking the receptors are also toxic to
10 animals. As such, there is currently no known effective treatment for glutamate abnormalities.

Prostate Cancer

 Prostate cancer is the leading form of cancer and the second leading cause of death from cancer for men in
15 the United States. The American Cancer Society has estimated that in 1996 alone, 317,100 new cases of prostate cancer were diagnosed and 41,400 deaths were caused by prostate cancer. The incidence rate of prostate cancer increased 65% between 1980 and 1990, and
20 will continue to rise with improved screening tests and longer life expectancies. While most men used to die of other illnesses before prostate cancer had a chance to develop, higher prostate cancer mortality rates are expected as men live longer and the disease has more time
25 to progress.

 In 1993, the molecular cloning of Prostate Specific

Membrane Antigen (PSMA) was reported as a potential prostate carcinoma marker and hypothesized to serve as a target for imaging and cytotoxic treatment modalities for prostate cancer. PSMA antibodies, particularly indium-111 labelled and itrium labelled PSMA antibodies, have been described and examined clinically for the diagnosis and treatment of prostate cancer. PSMA is expressed in prostatic ductal epithelium and is present in seminal plasma, prostatic fluid and urine. In 1996, it was found that the expression of PSMA cDNA confers the activity of NAALADase.

NAALADase Inhibitors

NAAG and NAALADase have been implicated in several human and animal pathological conditions. For example, it has been demonstrated that intra-hippocampal injections of NAAG elicit prolonged seizure activity. More recently, it was reported that rats genetically prone to epileptic seizures have a persistent increase in their basal level of NAALADase activity. These observations support the hypothesis that increased availability of synaptic glutamate elevates seizure susceptibility, and suggest that NAALADase inhibitors may provide anti-epileptic activity.

NAAG and NAALADase have also been implicated in the pathogenesis of ALS and in the pathologically similar animal disease called Hereditary Canine Spinal Muscular

Atrophy (HCSMA). It has been shown that concentrations of NAAG and its metabolites -- NAA, glutamate and aspartate -- are elevated two- to three-fold in the cerebrospinal fluid of ALS patients and HCSMA dogs. Additionally, NAALADase activity is significantly increased (two- to three-fold) in post-mortem spinal cord tissue from ALS patients and HCSMA dogs. As such, NAALADase inhibitors may be clinically useful in curbing the progression of ALS if increased metabolism of NAAG is responsible for the alterations of CSF levels of these acidic amino acids and peptides.

Abnormalities in NAAG levels and NAALADase activity have also been documented in post-mortem schizophrenic brain, specifically in the prefrontal and limbic brain regions.

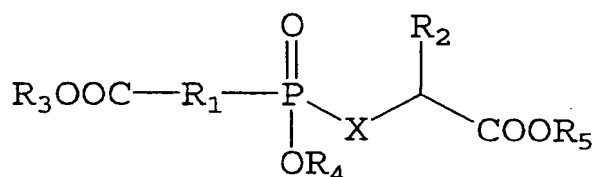
The findings described above suggest that NAALADase inhibitors could be useful in treating glutamate abnormalities. In fact, the results of studies conducted by the inventors confirm that NAALADase inhibitors are effective in treating glutamate abnormalities (particularly stroke, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), spinal cord injury, alcoholism and nicotine dependence), as well as prostate diseases (particularly prostate cancer).

While a few NAALADase inhibitors have been identified, they have only been used in non-clinical

research. Examples of such inhibitors include general metallopeptidase inhibitors such as o-phenanthroline, metal chelators such as EGTA and EDTA, and peptide analogs such as quisqualic acid and β -NAAG. Accordingly,
 5 a need exists for new NAALADase inhibitors, as well as pharmaceutical compositions and methods using such new and known NAALADase inhibitors to treat glutamate abnormalities and prostate diseases.

SUMMARY OF THE INVENTION

The present invention relates to a compound of formula I:



I

or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

20 X is CR_6R_7 , O or NR_8 ;

R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or
 25 substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl,

C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and
5 Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇
10 cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro,
15 trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently
20 selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain
25

alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

The present invention also relates to a method of treating a glutamate abnormality in an animal, comprising administering to said animal an effective amount of a compound of formula I.

The present invention further relates to a method of effecting a neuronal activity in an animal, comprising administering to said animal an effective amount of a compound of formula I.

Additionally, the present invention relates to a method of treating a compulsive disorder, comprising administering to a patient in need thereof an effective amount of a compound of formula I.

Furthermore, the present invention relates to a method of treating a prostate disease in an animal, comprising administering to said animal an effective amount of a compound of formula I.

Moreover, the present invention relates to a method of inhibiting NAALADase activity in an animal, comprising administering to said animal an effective amount of a compound of formula I.

Finally, the present invention relates to a pharmaceutical composition comprising:

(i) an effective amount of a compound of formula I;

and

(ii) a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar graph plotting *in vitro* toxicity of
5 ischemic insult (potassium cyanide and 2-deoxyglucose)
against various doses of 2-(phosphonomethyl)pentanedioic
acid with which cortical cell cultures were treated.

FIG. 2 is a bar graph plotting *in vitro* toxicity
against various doses of NAAG to which cortical cell
10 cultures were exposed.

FIG. 3 is a bar graph plotting *in vitro* toxicity
following treatment with 2-(phosphonomethyl)pentanedioic
acid, against various doses of NAAG to which cortical
cell cultures were exposed.

15 FIG. 4 is a bar graph plotting *in vitro* toxicity of
ischemic insult against various times at which cortical
cell cultures were treated with 2-(phosphonomethyl)-
pentanedioic acid.

FIG. 5 is a bar graph plotting *in vivo* cortical
20 injury volume against various doses of 2-
(phosphonomethyl)pentanedioic acid with which rats were
treated after sustaining middle cerebral artery
occlusion.

FIG. 6 is a bar graph plotting *in vivo* total brain
25 infarct volume of rats against various times at which the
rats are treated with 2-(phosphonomethyl)pentanedioic

acid after sustaining middle cerebral artery occlusion.

FIG. 7 is a bar graph plotting *in vivo* extracellular glutamate increases in the striatum of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

FIG. 8 is a bar graph plotting *in vivo* extracellular glutamate increases in the parietal cortex of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

FIG. 9 is a bar graph plotting *in vivo* extracellular glutamate increases in the frontal cortex of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

FIG. 10(a) is a photomicrograph of mouse sciatic nerve treated with a vehicle following cryolesion.

FIG. 10(b) is a photomicrograph of mouse sciatic nerve treated with 2-(phosphonomethyl)pentanedioic acid following cryolesion.

FIG. 11 is a bar graph plotting percent striatal TH innervation density against the treatment of mice with vehicle alone, vehicle following MPTP, or 2-(phosphonomethyl)pentanedioic acid following MPTP.

FIG. 12 is a bar graph plotting the neurological function code against the treatment of rats with dynorphin A alone or 2-(phosphonomethyl)pentanedioic acid with dynorphin A.

FIG. 13 is a bar graph plotting the ChAT activity of rat spinal cord organotypic cultures against the treatment of the cultures with 2-(phosphonomethyl)-pentanedioic acid alone, threohydroxyaspartate (THA) alone, or THA with 2-(phosphonomethyl)pentanedioic acid.

FIG. 14 is a bar graph plotting the ChAT activity of rat spinal cord organotypic cultures against various doses of 2-(phosphonomethyl)pentanedioic acid with which the cultures were treated in the presence of THA.

FIG. 15 is a bar graph plotting the ethanol intake of alcohol-preferring rats against various doses of 2-(phosphonomethyl)pentanedioic acid with which the rats were treated.

FIG. 16 is a graph plotting the cumulative nicotine intake of rats during a 1 hour test session, before which the rats had been trained to self-administer nicotine and pretreated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid.

FIG. 17 is a graph plotting the cumulative food intake of rats during a 1 hour test session, before which the rats had been trained to self-administer nicotine and pretreated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid.

FIG. 18 is a bar graph plotting *in vitro* cancer cell growth against various doses of quisqualic acid with which LNCAP cells were treated.

FIG. 19 is a bar graph plotting *in vitro* cancer cell growth against various doses of 2-(phosphonomethyl)-pentanedioic acid with which LNCAP cells were treated.

5

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Attention Deficit Disorder" refers to a disorder characterized by developmentally inappropriate inattention and impulsivity, with or without hyperactivity. Inattention means a failure to finish tasks started, easy distractibility, seeming lack of attention, and difficulty concentrating on tasks requiring sustained attention. Impulsivity means acting before thinking, difficulty taking turns, problems organizing work, and constant shifting from one activity to another. Hyperactivity means difficulty staying seated and sitting still, and running or climbing excessively.

"Compound 3" refers to 2-(phosphonomethyl)pentanedioic acid (PMPA).

"Compulsive disorder" refers to any disorder characterized by irresistible impulsive behavior. Examples of compulsive disorders include without limitation drug dependence, eating disorders, pathological gambling, ADD and Tourette's syndrome.

"Drug dependence" refers to a psychologic addiction

or a physical tolerance to a drug. Tolerance means a need to increase the dose progressively in order to produce the effect originally achieved by smaller amounts.

5 "Eating disorder" refers to compulsive overeating, obesity or severe obesity. Obesity means body weight of 20% over standard height-weight tables. Severe obesity means over 100% overweight.

10 "Glutamate abnormality" refers to any disease, disorder or condition in which glutamate is implicated, including pathological conditions involving elevated levels of glutamate. Examples of glutamate abnormalities include epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS),
15 Huntington's Disease, schizophrenia, chronic pain, ischemia, neuronal insult and compulsive disorders.

 "Glutamate modulator" refers to any composition of matter which alone or in combination with another agent affects the level of glutamate in an animal.

20 "Inhibition", in the context of enzymes, refers to reversible enzyme inhibition such as competitive, uncompetitive and non-competitive inhibition. Competitive, uncompetitive and non-competitive inhibition can be distinguished by the effects of an inhibitor on
25 the reaction kinetics of an enzyme. Competitive inhibition occurs when the inhibitor combines reversibly

with the enzyme in such a way that it competes with a normal substrate for binding at the active site. The affinity between the inhibitor and the enzyme may be measured by the inhibitor constant, K_i , which is defined as:

$$K_i = \frac{[E][I]}{[EI]}$$

10

wherein [E] is the concentration of the enzyme, [I] is the concentration of the inhibitor, and [EI] is the concentration of the enzyme-inhibitor complex formed by the reaction of the enzyme with the inhibitor. Unless otherwise specified, K_i as used herein refers to the affinity between the inventive compounds and NAALADase. "IC₅₀" is a related term used to define the concentration or amount of a compound which is required to cause a 50% inhibition of the target enzyme.

"Ischemia" refers to localized tissue anemia due to obstruction of the inflow of arterial blood. Global ischemia occurs when blood flow to the entire brain ceases for a period of time, such as may result from cardiac arrest. Focal ischemia occurs when a portion of the brain is deprived of its normal blood supply, such as may result from thromboembolytic occlusion of a cerebral vessel, traumatic head injury, edema or brain tumor. Even if transient, both global and focal ischemia can

produce widespread neuronal damage. Although nerve tissue damage occurs over hours or even days following the onset of ischemia, some permanent nerve tissue damage may develop in the initial minutes following cessation of blood flow to the brain. Much of this damage is attributed to glutamate toxicity and secondary consequences of reperfusion of the tissue, such as the release of vasoactive products by damaged endothelium, and the release of cytotoxic products, such as free radicals and leukotrienes, by the damaged tissue.

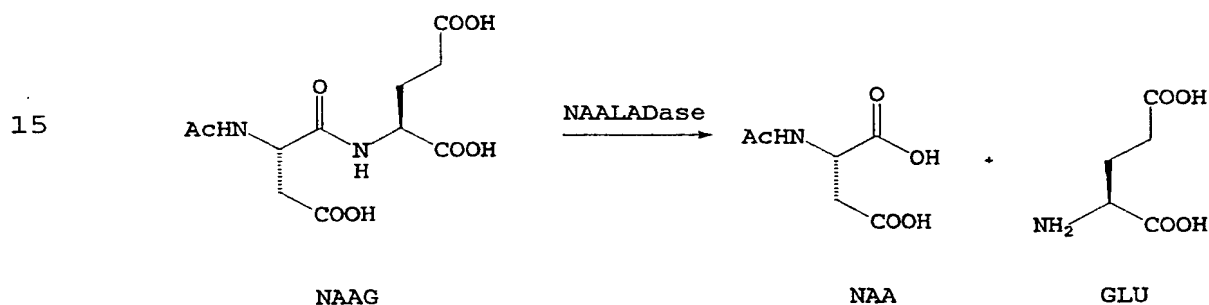
"Isomers" refer to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the arrangement or configuration of the atoms. "Stereoisomers" are isomers that differ only in the arrangement of the atoms in space. "Enantiomers" are a pair of stereoisomers that are non-superimposable mirror images of each other. "Diastereoisomers" are stereoisomers which are not mirror images of each other. "Racemic mixture" means a mixture containing equal parts of individual enantiomers. "Non-racemic mixture" is a mixture containing unequal parts of individual enantiomers or stereoisomers.

"NAAG" refers to N-acetyl-aspartyl-glutamate, an important peptide component of the brain, with levels comparable to the major inhibitor neurotransmitter gamma-aminobutyric acid (GABA). NAAG is neuron-specific,

present in synaptic vesicles and released upon neuronal stimulation in several systems presumed to be glutamatergic. Studies suggest that NAAG may function as a neurotransmitter and/or neuromodulator in the central nervous system, or as a precursor of the neurotransmitter glutamate.

"NAALADase" refers to N-acetylated α -linked acidic dipeptidase, a membrane-bound metalloproteinase which catabolizes NAAG to N-acetylaspartate (NAA) and glutamate:

Catabolism of NAAG by NAALADase



20

NAALADase shows a high affinity for NAAG with a K_m of 540 nM. If NAAG is a bioactive peptide, then NAALADase may serve to inactivate NAAG'S synaptic action. Alternatively, if NAAG functions as a precursor for glutamate, the primary function of NAALADase may be to regulate synaptic glutamate availability.

"Nervous function" refers to the various functions of the nervous system, which among other things provide an awareness of the internal and external environments of the body, make possible voluntary and reflex activities between the various structural elements of the organism, and balance the organism's response to environmental changes.

"Nervous insult" refers to any damage to nervous tissue and any disability or death resulting therefrom. The cause of nervous insult may be metabolic, toxic, neurotoxic, iatrogenic, thermal or chemical, and includes without limitation ischemia, hypoxia, cerebrovascular accident, trauma, surgery, pressure, mass effect, hemorrhage, radiation, vasospasm, neurodegenerative disease, neurodegenerative process, infection, Parkinson's disease, ALS, myelination/demyelination process, epilepsy, cognitive disorder, glutamate abnormality and secondary effects thereof. Currently, there is no known effective treatment for nervous tissue damage.

"Nervous tissue" refers to the various components that make up the nervous system, including without limitation neurons, neural support cells, glia, Schwann cells, vasculature contained within and supplying these structures, the central nervous system, the brain, the brain stem, the spinal cord, the junction of the central

nervous system with the peripheral nervous system, the peripheral nervous system and allied structures.

"Neuroprotective" refers to the effect of reducing, arresting or ameliorating nervous insult, and protecting, resuscitating or reviving nervous tissue which has suffered nervous insult.

"Pathological gambling" is a condition characterized by a preoccupation with gambling. Similar to psychoactive substance abuse, its effects include development of tolerance with a need to gamble progressively larger amounts of money, withdrawal symptoms, and continued gambling despite severe negative effects on family and occupation.

"Pharmaceutically acceptable salt" refers to a salt of the inventive compounds which possesses the desired pharmacological activity and which is neither biologically nor otherwise undesirable. The salt can be formed with inorganic acids such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate,

oxalate, thiocyanate, tosylate and undecanoate. Examples of a base salt include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine. The basic nitrogen-containing groups can be quarternized with agents including lower alkyl halides such as methyl, ethyl, propyl and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

"Pharmaceutically acceptable prodrug" refers to a derivative of the inventive compounds which undergoes biotransformation prior to exhibiting its pharmacological effect(s). The prodrug is formulated with the objective(s) of improved chemical stability, improved patient acceptance and compliance, improved bioavailability, prolonged duration of action, improved organ selectivity, improved formulation (e.g., increased hydrosolubility), and/or decreased side effects (e.g., toxicity). The prodrug can be readily prepared from the inventive compounds using methods known in the art, such as those described by *Burger's Medicinal Chemistry and*

Drug Chemistry, Fifth Ed., Vol. 1, pp. 172-178, 949-982 (1995). For example, the inventive compounds can be transformed into prodrugs by converting one or more of the hydroxy or carboxy groups into esters.

5 "Tourette's syndrome" refers to an autosomal multiple tic disorder characterized by compulsive swearing, multiple muscle tics and loud noises. Tics are brief, rapid, involuntary movements that can be simple or complex; they are stereotyped and repetitive, but not
10 rhythmic. Simple tics, such as eye blinking, often begin as nervous mannerisms. Complex tics often resemble fragments of normal behavior.

"Treating" refers to:

(i) preventing a disease, disorder or condition
15 from occurring in an animal which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and

20 (iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

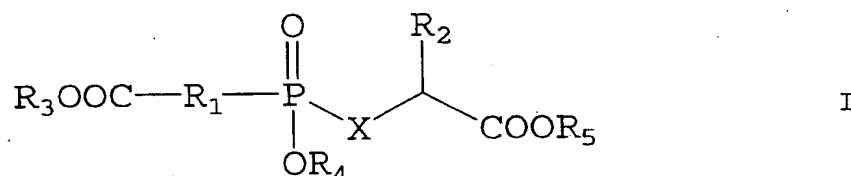
In relation to drug dependence, "treating" refers to suppressing the psychologic addiction or physical
25 tolerance to the drug of abuse, and relieving or preventing a withdrawal syndrome resulting from the drug

dependence.

"Withdrawal syndrome" refers to a disorder characterized by untoward physical changes that occur when the drug is discontinued or when its effect is counteracted by a specific antagonist.

COMPOUNDS OF THE PRESENT INVENTION

The present invention relates to a compound of formula I:



15 or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉

alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

In a preferred embodiment, X is CH₂.

In a more preferred embodiment, R_2 is $-(CH_2)_2COOR_9$; and R_9 is selected from the group consisting of hydrogen, C_1-C_9 straight or branched chain alkyl, C_2-C_9 straight or branched chain alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl and Ar, wherein said R_9 is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 straight or branched chain alkyl, C_2-C_6 straight or branched chain alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

In the most preferred embodiment, R_3 , R_4 , R_5 , and R_9 are hydrogen.

Preferred compounds of formula I are selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxybutyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl]methyl]-
pentanedioic acid;

2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl]methyl]pentanedioic acid;

5 2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl]methyl]pentanedioic acid;

2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxy-
phosphinyl]methyl]pentanedioic acid;

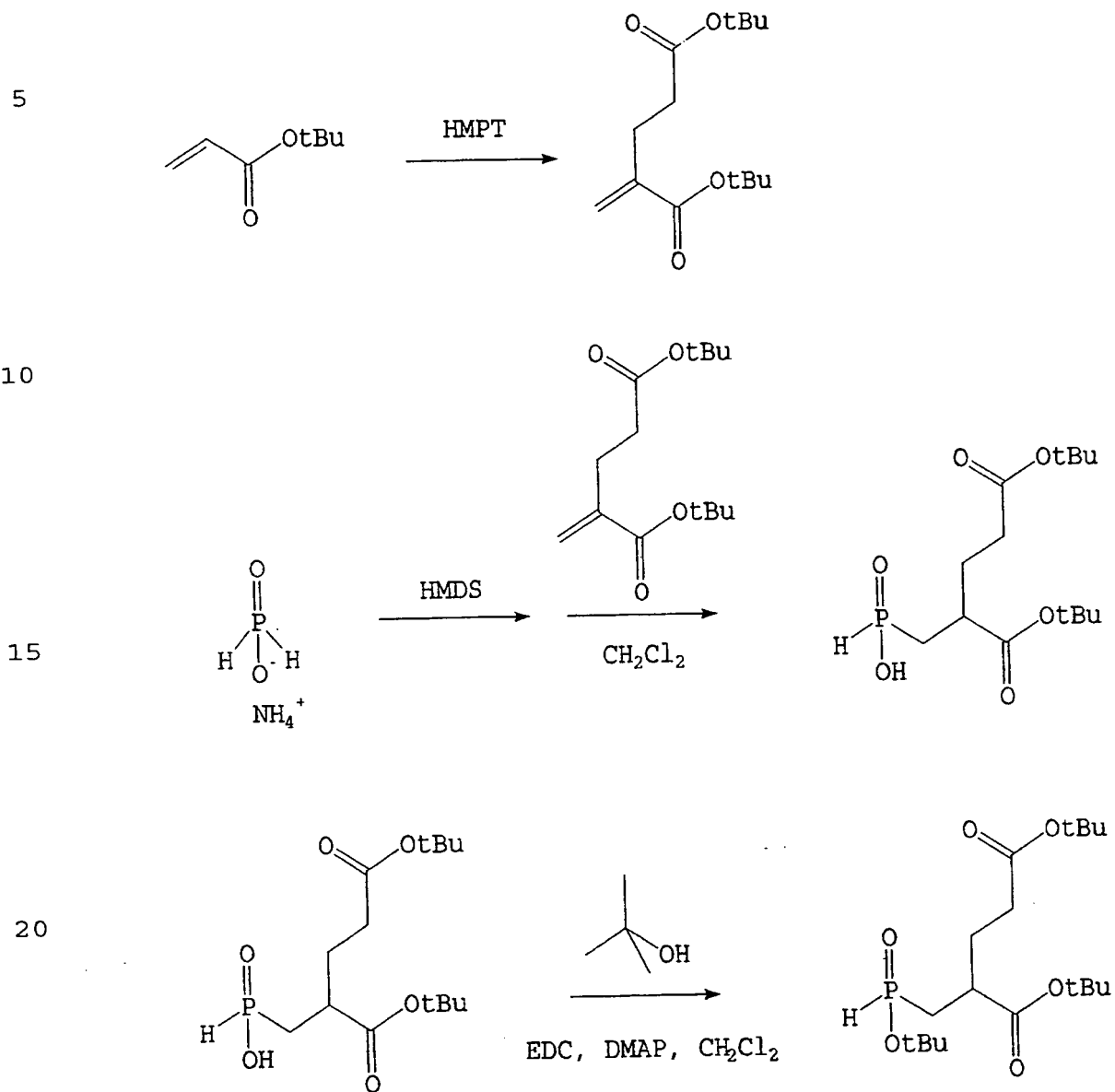
10 2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-
phosphinyl]methyl]pentanedioic acid; and
pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

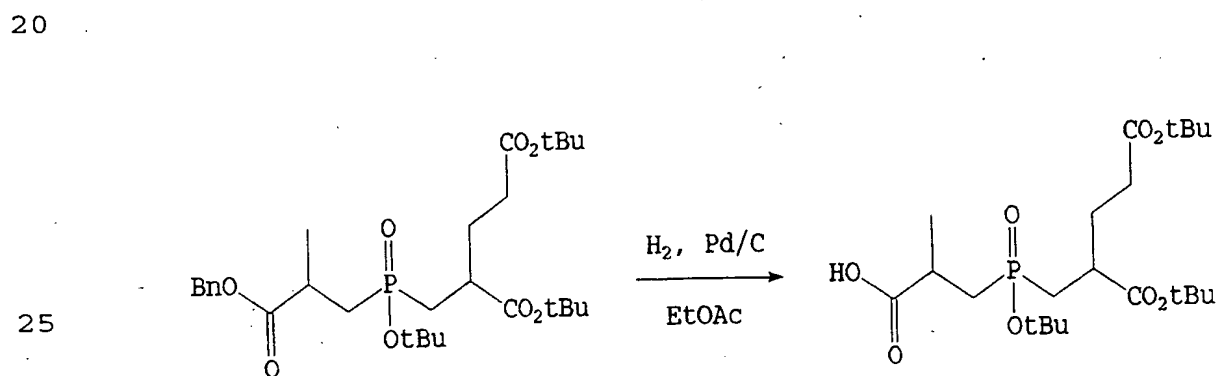
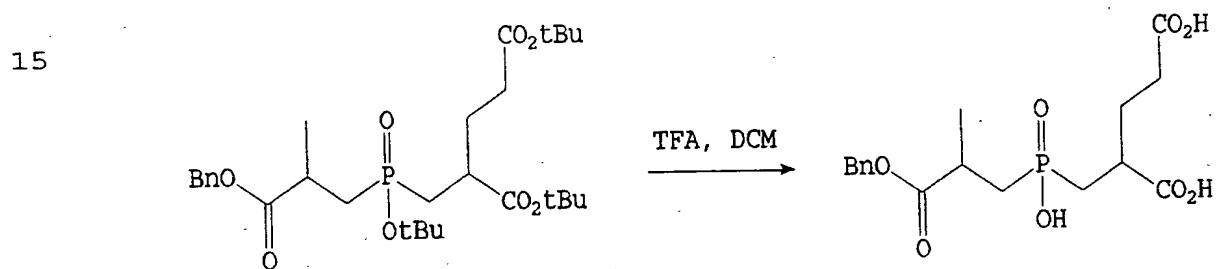
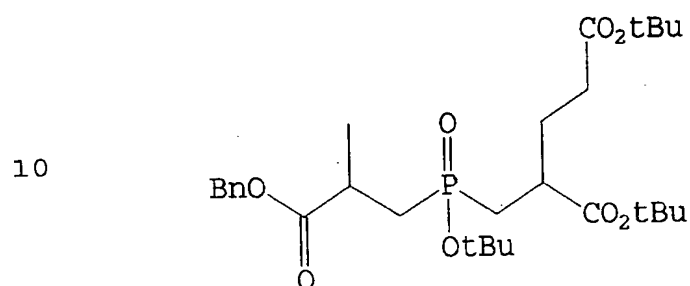
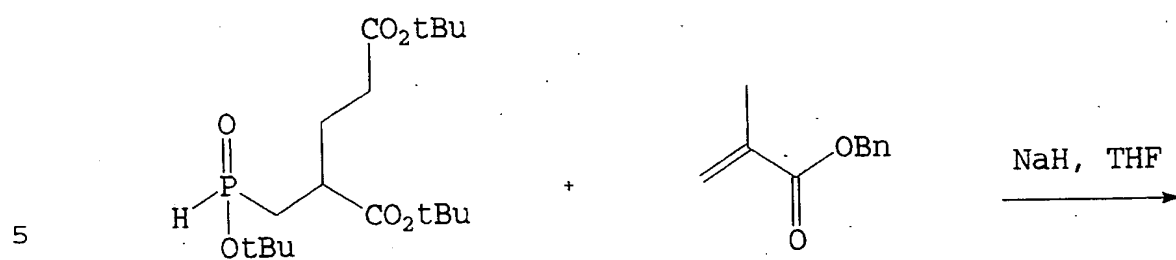
The most preferred compound of formula I is 2-[[[(2-
carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid,
15 or a pharmaceutically acceptable salt, hydrate or prodrug
thereof.

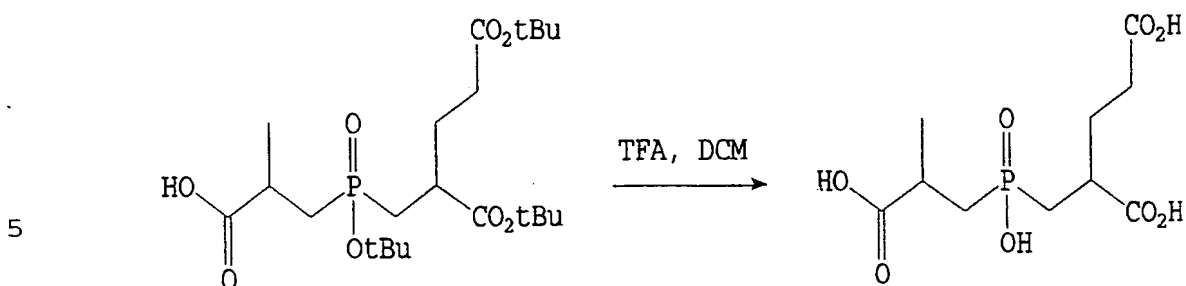
The compounds of the present invention possess one
or more asymmetric center(s) and thus can be produced as
mixtures (racemic and non-racemic) of stereoisomers, or
20 as individual R- and S-stereoisomers. The individual
stereoisomers may be obtained by using an optically
active starting material, by resolving a racemic or non-
racemic mixture of an intermediate at some appropriate
stage of synthesis, or by resolving a compound of formula
25 I.

Synthesis of Compounds

The compounds of the present invention can be readily prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways depicted below in Scheme I. Precursor compounds can be prepared by methods known in the art, such as those described by Jackson et al., *J. Med. Chem.*, Vol. 39, No. 2, pp. 619-622 (1996) and Froestl et al., *J. Med. Chem.*, Vol. 38, pp. 3313-3331 (1995).

Scheme I





10 PHARMACEUTICAL COMPOSITIONS OF THE PRESENT INVENTION

The present invention also relates to a pharmaceutical composition comprising:

- (i) an effective amount of the compound of formula I; and
- 15 (ii) a pharmaceutically acceptable carrier.

Examples of the compound of formula I are set forth above.

METHODS OF THE PRESENT INVENTION

20

METHOD OF INHIBITING NAALADASE ENZYME ACTIVITY

The present invention further relates to a method of inhibiting NAALADase enzyme activity in an animal, comprising administering an effective amount of the compound of formula I to said animal.

25

METHOD OF TREATING GLUTAMATE ABNORMALITY

Although not limited to any one particular theory,
it is believed that the compounds of the present
invention modulate levels of glutamate by acting on a
5 storage form of glutamate which is hypothesized to be
upstream from the effects mediated by the NMDA receptor.

Accordingly, the present invention further relates
to a method of treating a glutamate abnormality in an
animal, comprising administering an effective amount of
10 a prodrug of the compound of formula I to said animal.

The glutamate abnormality may be any disease,
disorder or condition in which glutamate is implicated,
including pathological conditions involving elevated
levels of glutamate. Examples of glutamate abnormalities
15 include without limitation epilepsy, stroke, Alzheimer's
disease, Parkinson's Disease, Amyotrophic Lateral
Sclerosis (ALS), Huntington's Disease, schizophrenia,
chronic pain, ischemia, peripheral neuropathy, traumatic
brain injury and physical damage to the spinal cord. In
20 a preferred embodiment, the glutamate abnormality is
selected from the group consisting of ischemia, stroke,
Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS)
and spinal cord injury.

25

METHOD OF TREATING COMPULSIVE DISORDER

The inventors have unexpectedly found that the compounds of the present invention are effective in treating glutamate-related compulsive disorders.

5 Accordingly, the present invention also relates to a method of treating a compulsive disorder, comprising administering an effective amount of the compound of formula I to a patient in need thereof.

10 The compulsive disorder may be any disorder characterized by irresistible impulsive behavior. Examples of compulsive disorders treatable by the methods of the present invention include drug dependence, eating disorders, pathological gambling, ADD and Tourette's syndrome.

15 Preferably, the compulsive disorder is drug dependence. Commonly used drugs with potential for dependence include CNS depressants (opioids, synthetic narcotics, barbiturates, glutethimide, methyprylon, ethchlorvynol, methaqualone, alcohol); anxiolytics
20 (diazepam, chlordiazepoxide, alprazolam, oxazepam, temazepam); stimulants (amphetamine, methamphetamine, cocaine); and hallucinogens (LSD, mescaline, peyote, marijuana).

25 More preferably, the drug dependence is alcohol, nicotine, heroin or cocaine dependence.

METHOD OF EFFECTING NEURONAL ACTIVITY

The inventors have also discovered that inhibition of NAALADase promotes nerve regeneration and myelin formation.

5 Accordingly, the present invention further relates to a method of effecting a neuronal activity in an animal; comprising administering an effective amount of the compound of formula I to said animal.

10 The neuronal activity that is effected by the method of the present invention may be selected from the group consisting of: stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration and treatment of a neurological disorder.

15 Examples of a neurological disorder that is treatable by the method of the present invention include without limitation: trigeminal neuralgia; glossopharyngeal neuralgia; Bell's Palsy; myasthenia gravis; muscular dystrophy; amyotrophic lateral sclerosis; progressive muscular atrophy; progressive
20 bulbar inherited muscular atrophy; herniated, ruptured or prolapsed invertebrate disk syndromes; cervical spondylosis; plexus disorders; thoracic outlet destruction syndromes; peripheral neuropathies such as those caused by lead, dapsone, ticks, porphyria, or
25 Guillain-Barré syndrome; Alzheimer's disease; and Parkinson's disease.

The method of the present invention is particularly useful for treating a neurological disorder selected from the group consisting of: peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke associated with brain damage, demyelinating diseases and neurological disorders relating to neurodegeneration. Examples of demyelinating diseases include multiple sclerosis. Examples of neurological disorders relating to neurodegeneration include Alzheimer's Disease, Parkinson's Disease, and amyotrophic lateral sclerosis (ALS).

METHOD OF TREATING PROSTATE DISEASE

Additionally, the present invention relates to a method of treating a prostate disease in an animal, comprising administering an effective amount of the compound of formula I to said animal.

In a preferred embodiment, said prostate disease is prostate cancer or benign prostatic hyperplasia.

METHOD OF TREATING CANCER

In addition to prostate cancer, other forms of cancer that may be treated with the compounds of the present invention include without limitation: ACTH-producing tumors, acute lymphocytic leukemia, acute

nonlymphocytic leukemia, cancer of the adrenal cortex,
bladder cancer, brain cancer, breast cancer, cervix
cancer, chronic lymphocytic leukemia, chronic myelocytic
leukemia, colorectal cancer, cutaneous T-cell lymphoma,
5 endometrial cancer, esophageal cancer, Ewing's sarcoma,
gallbladder cancer, hairy cell leukemia, head & neck
cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney
cancer, liver cancer, lung cancer (small and/or non-small
cell), malignant peritoneal effusion, malignant pleural
10 effusion, melanoma, mesothelioma, multiple myeloma,
neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma,
ovary cancer, ovary (germ cell) cancer, pancreatic
cancer, penis cancer, retinoblastoma, skin cancer, soft-
tissue sarcoma, squamous cell carcinomas, stomach cancer,
15 testicular cancer, thyroid cancer, trophoblastic
neoplasms, cancer of the uterus, vaginal cancer, cancer
of the vulva and Wilm's tumor.

The compounds of the present invention are
particularly useful in treating cancer of tissues where
20 NAALADase enzymes reside. Such tissues include the
prostate as well as the brain, kidney and testis.

ROUTE OF ADMINISTRATION

In the methods of the present invention, the
25 compounds may be administered orally, parenterally, by
inhalation spray, topically, rectally, nasally, buccally,

vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes
5 subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal or intracranial injection and infusion techniques. Invasive techniques are preferred, particularly direct administration to damaged neuronal
10 tissue.

To be effective therapeutically as central nervous system targets, the compounds of the present invention should readily penetrate the blood-brain barrier when peripherally administered. Compounds which cannot
15 penetrate the blood-brain barrier can be effectively administered by an intraventricular route.

The compounds may also be administered in the form of sterile injectable preparations, for example, as sterile injectable aqueous or oleaginous suspensions.
20 These suspensions can be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparations may also be sterile injectable solutions or suspensions in non-toxic parenterally-
25 acceptable diluents or solvents, for example, as solutions in 1,3-butanediol. Among the acceptable

vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils are conventionally employed as solvents or suspending mediums. For this purpose, any bland fixed oil such as a synthetic mono- or di-glyceride may be employed. Fatty acids such as oleic acid and its glyceride derivatives, including olive oil and castor oil, especially in their polyoxyethylated forms, are useful in the preparation of injectables. These oil solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

Additionally, the compounds may be administered orally in the form of capsules, tablets, aqueous suspensions or solutions. Tablets may contain carriers such as lactose and corn starch, and/or lubricating agents such as magnesium stearate. Capsules may contain diluents including lactose and dried corn starch. Aqueous suspensions may contain emulsifying and suspending agents combined with the active ingredient. The oral dosage forms may further contain sweetening and/or flavoring and/or coloring agents.

The compounds may further be administered rectally in the form of suppositories. These compositions can be prepared by mixing the drug with suitable non-irritating excipients which are solid at room temperature, but liquid at rectal temperature such that they will melt in

the rectum to release the drug. Such excipients include cocoa butter, beeswax and polyethylene glycols.

Moreover, the compounds may be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin or the lower intestinal tract.

For topical application to the eye, or ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline or, preferably, as a solution in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, the compounds may be formulated into ointments, such as petrolatum.

For topical application to the skin, the compounds can be formulated into suitable ointments containing the compounds suspended or dissolved in, for example, mixtures with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated into suitable lotions or creams containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax,

cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application to the lower intestinal tract can be effected in rectal suppository formulations (see above) or in suitable enema formulations.

The compounds of the present invention may be administered by a single dose, multiple discrete doses or continuous infusion. Since the compounds are small, easily diffusible and relatively stable, they are well suited to continuous infusion. Pump means, particularly subcutaneous pump means, are preferred for continuous infusion.

DOSAGE

Dose levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels being about 0.1 mg to about 1,000 mg. The specific dose level for any particular patient will vary depending upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the rate of excretion; drug combination; the severity of the particular disease being treated; and the form of administration. Typically, in vitro dosage-effect results provide useful guidance on

the proper doses for patient administration. Studies in animal models are also helpful. The considerations for determining the proper dose levels are well known in the art.

5 In a preferred embodiment, the compounds are administered in lyophilized form. In this case, 1 to 100 mg of a compound of the present invention may be lyophilized in individual vials, together with a carrier and a buffer, such as mannitol and sodium phosphate. The
10 compound may be reconstituted in the vials with bacteriostatic water before administration.

 In treating global ischemia, the compounds of the present invention are preferably administered orally, rectally, parenterally or topically at least 1 to 6 times
15 daily, and may follow an initial bolus dose of higher concentration.

 The compounds of the present invention may be administered in combination with one or more therapeutic agents, including chemo-therapeutic agents. TABLE I
20 provides known median dosages for selected chemotherapeutic agents. Specific dose levels for these agents and other therapeutic agents will depend upon considerations such as those identified above for the compounds of the present invention.

TABLE I

	CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
	Asparaginase	10,000 units
	Bleomycin Sulfate	15 units
5	Carboplatin	50-450 mg
	Carmustine	100 mg
	Cisplatin	10-50 mg
	Cladribine	10 mg
10	Cyclophosphamide (lyophilized)	100 mg-2 gm
	Cyclophosphamide (non-lyophilized)	100 mg-2 gm
	Cytarabine (lyophilized powder)	100 mg-2 gm
15	Dacarbazine	100 mg-200 mg
	Dactinomycin	0.5 mg
	Daunorubicin	20 mg
	Diethylstilbestrol	250 mg
	Doxorubicin	10-150 mg
20	Etidronate	300 mg
	Etoposide	100 mg
	Floxuridine	500 mg
	Fludarabine Phosphate	50 mg
	Fluorouracil	500 mg-5 gm
25	Goserelin	3.6 mg
	Granisetron Hydrochloride	1 mg
	Idarubicin	5-10 mg
	Ifosfamide	1-3 gm
30	Leucovorin Calcium	50-350 mg
	Leuprolide	3.75-7.5 mg
	Mechlorethamine	10 mg

	CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
	Medroxyprogesterone	1 gm
	Melphalan	50 gm
	Methotrexate	20 mg-1 gm
	Mitomycin	5-40 mg
5	Mitoxantrone	20-30 mg
	Ondansetron Hydrochloride	40 mg
	Paclitaxel	30 mg
	Pamidronate Disodium	30-*90 mg
10	Pegaspargase	750 units
	Plicamycin	2,500 mcgm
	Streptozocin	1 gm
	Thiotepa	15 mg
	Teniposide	50 mg
15	Vinblastine	10 mg
	Vincristine	1-5 mg
	Aldesleukin	22 million units
	Epoetin Alfa	2,000-10,000 units
	Filgrastim	300-480 mcgm
20	Immune Globulin	500 mg-10 gm
	Interferon Alpha-2a	3-36 million units
	Interferon Alpha-2b	3-50 million units
	Levamisole	50 mg
	Octreotide	1,000-5,000 mcgm
25	Sargramostim	250-500 mcgm

ADMINISTRATION REGIMEN

For the methods of the present invention, any administration regimen regulating the timing and sequence of drug delivery can be used and repeated as necessary to

effect treatment. Such regimen may include pretreatment and/or co-administration with additional therapeutic agents.

To maximize protection of nervous tissue from nervous insult, the compounds should be administered to the affected cells as soon as possible. In situations where nervous insult is anticipated, the compounds should be administered before the expected nervous insult. Such situations of increased likelihood of nervous insult include surgery (cardiovascular, cardiac, vascular, aortic, orthopedic); endovascular procedures such as arterial catheterization (cardiovascular, vertebral, aortic, cardiac, renal, spinal, Adamkiewicz); injections of embolic agents; coils or balloons for hemostasis; interruptions of vascularity for treatment of brain lesions; and predisposing medical conditions such as crescendo transient ischemic attacks, emboli and sequential strokes. Where pretreatment for stroke or ischemia is impossible or impracticable, it is important to get the compounds to the affected cells as soon as possible during or after the event. In the time period between strokes, diagnosis and treatment procedures should be minimized to save the cells from further damage and death.

For patients with prostate cancer that is neither advanced nor metastatic, the compounds of the present

invention may be administered (i) prior to surgery or radiation treatment to reduce the risk of metastasis; (ii) during surgery or in conjunction with radiation treatment; and/or (iii) after surgery or radiation therapy to reduce the risk of recurrence and to inhibit the growth of any residual tumorous cells.

For patients with advanced or metastatic prostate cancer, the compounds of the present invention may be administered as a continuous supplement to, or as a replacement for, hormonal ablation in order to slow tumor cell growth in both the untreated primary tumor and the existing metastatic lesions.

The methods of the present invention are particularly useful where shed cells could not be removed by surgical intervention. After post-surgical recovery, the methods of the present invention would be effective in reducing the chances of recurrence of a tumor engendered by such shed cells.

COMBINATION WITH OTHER TREATMENTS

a. Nervous Insult

In methods of treating nervous insult (particularly acute ischemic stroke and global ischemia caused by drowning and head trauma), the compounds of the present invention can be co-administered with one or more therapeutic agents, preferably agents which can reduce

the risk of stroke (such as aspirin), and more preferably agents which can reduce the risk of a second ischemic event (such as ticlopidine).

5 The compounds of the present invention can be co-administered with one or more therapeutic agents either (i) together in a single formulation, or (ii) separately in individual formulations designed for optimal release rates of their respective active agent. Each formulation may contain from about 0.01% to about 99.99% by weight, 10 preferably from about 3.5% to about 60% by weight, of a compound of the present invention, as well as one or more pharmaceutical excipients, such as wetting, emulsifying and pH buffering agents.

b. Prostate Disease

15 **(i) Surgery and Radiation Treatment**

In general, surgery and radiation treatment are employed as potentially curative therapies for patients with localized prostate cancer who are under 70 years of age and are expected to live at least 10 more years.

20 Approximately 70% of newly diagnosed prostate cancer patients fall into this category. Approximately 90% of these patients (65% of total patients) undergo surgery, while approximately 10% of these patients (7% of total patients) undergo radiation treatment.

25 Histopathological examination of surgical specimens reveals that approximately 63% of patients undergoing

surgery (40% of total patients) have locally extensive tumors or regional (lymph node) metastasis that was undetected at initial diagnosis. These patients are at a significantly greater risk of recurrence.

5 Approximately 40% of these patients will actually develop recurrence within five years after surgery. Results after radiation treatment are even less encouraging. Approximately 80% of patients who have undergone radiation treatment as their primary therapy have disease

10 persistence or develop recurrence or metastasis within five years after treatment.

Currently, most prostate cancer patients undergoing surgery and radiation treatment do not receive any immediate follow-up therapy. Rather, they are monitored

15 frequently for elevated Prostate Specific Antigen ("PSA"), which is the primary indicator of recurrence or metastasis.

Based on the above statistics, there is considerable opportunity to use the present invention in conjunction

20 with surgery and/or radiation treatment.

(ii) Hormonal Therapy

Hormonal ablation is the most effective palliative treatment for the 10% of patients with metastatic prostate cancer. Hormonal ablation by medication and/or

25 orchiectomy is used to block hormones that promote further growth and metastasis of prostate cancer. With

time, both the primary and metastatic tumors of virtually all of these patients become hormone-independent and resistant to therapy. Approximately 50% of patients with metastatic cancer die within three years after initial
5 diagnosis, and 75% of such patients die within five years after diagnosis. Continuous supplementation with the compounds of the present invention may be used to prevent or reverse this potentially metastasis-permissive state.

(iii) Chemotherapy

10. While chemotherapy has been successful in treating some forms of cancer, it has shown slight therapeutic value in treating prostate cancer where it is generally reserved as a last resort. Accordingly, the opportunity to treat prostate cancer by combining chemotherapy with
15 the methods of the present invention will be rare. When combined, however, such treatments should be more effective than chemotherapy alone in controlling prostate cancer.

(iv) Immunotherapy

20 The compounds of the present invention may also be used in combination with monoclonal antibodies to treat prostate cancer. Such combined treatment is particularly effective for patients with pelvic lymph node involvement, of which only 34% survive after 5 years. An
25 example of such monoclonal antibodies is cell membrane-specific anti-prostate antibody.

The present invention may also be used with immunotherapies based on polyclonal or monoclonal antibody-derived reagents. Monoclonal antibody-derived reagents are preferred. These reagents are well known in the art, and include radiolabelled monoclonal antibodies such as monoclonal antibodies conjugated with strontium-89.

(v) Cryotherapy

The methods of the present invention may also be used in conjunction with cryotherapy for treatment of prostate cancer.

In Vivo Toxicity of NAALADase Inhibitors

To examine the toxicological effect of NAALADase inhibition *in vivo*, a group of mice were injected with 2-(phosphonomethyl)pentanedioic acid, a NAALADase inhibitor of high activity, in doses of 1, 5, 10, 30, 100, 300 and 500 mg/kg body weight. The mice were subsequently observed two times per day for 5 consecutive days. The survival rate at each dose level is provided below in TABLE I. The results show that the NAALADase inhibitor is non-toxic to mice, suggesting that it would be similarly non-toxic to humans when administered at therapeutically effective amounts.

TABLE I

TOXICOLOGICAL EFFECTS OF NAALADASE INHIBITORS							
Dose (mg/kg)	1	5	10	30	100	300	500
Survival Rate After 5 days (%)	10 0	100	100	100	100	100	66.7

In Vitro Inhibition of NAALADase Activity

Various compounds of formula I were tested for in vitro inhibition of NAALADase activity. The results are provided below in Table II.

TABLE II
IN VITRO INHIBITION OF NAALADASE ACTIVITY

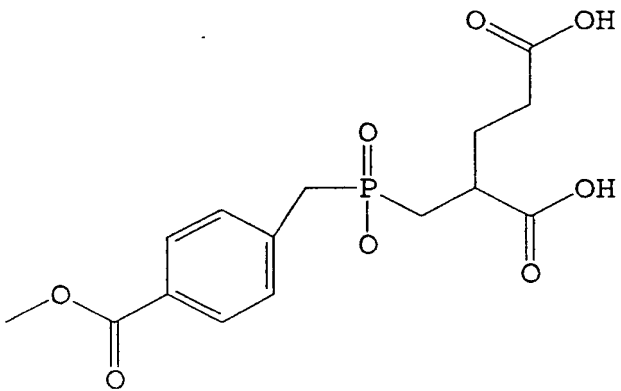
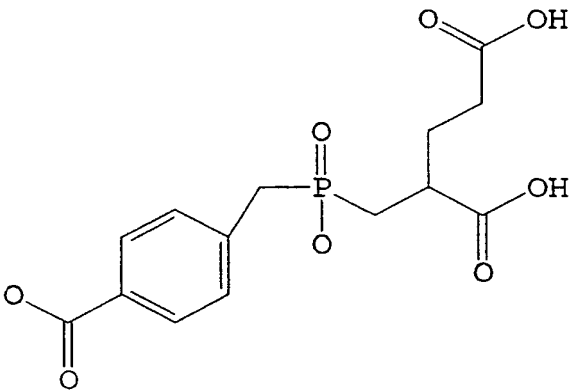
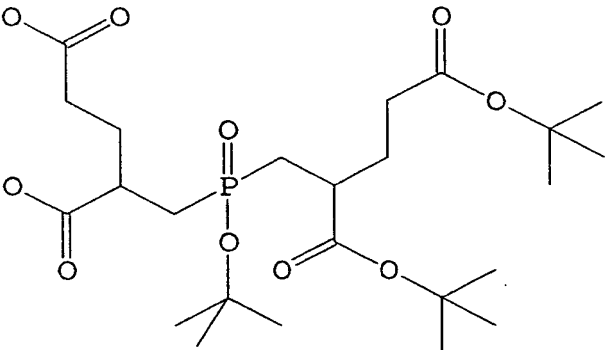
	Compound	K_i (nM)
5		
10		740.0000
15		198.5000
20		
25		4250.0000

TABLE II (continued)
IN VITRO INHIBITION OF NAALADASE ACTIVITY

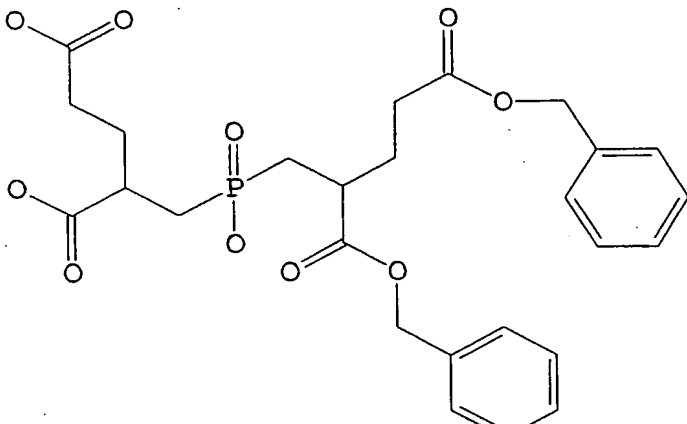
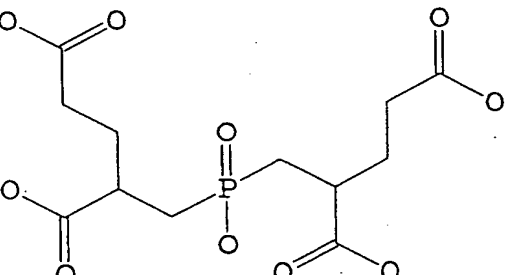
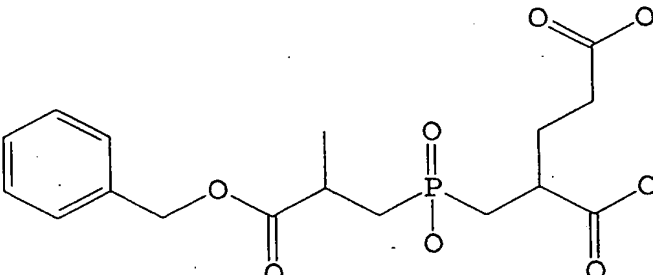
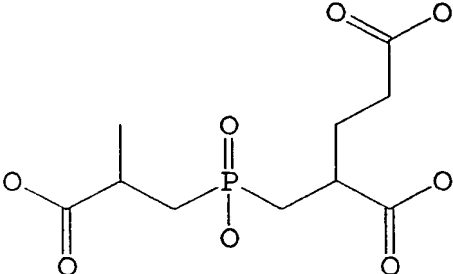
Compound	K_i (nM)
	12.6667
	0.5700
	95.0000

TABLE II (continued)
IN VITRO INHIBITION OF NAALADASE ACTIVITY

5	Compound	K_i (nM)
10		1.5000

Protocol for Assaying In Vitro Inhibition of NAALADase

15

Activity

The amount of [^3H]Glu liberated from [^3H]NAAG in 50 mM Tris-Cl buffer was measured for 15 minutes at 37° C using 30-50 μg of synaptosomal protein. Substrate and product were resolved by anion-exchange liquid chromatography. Duplicate assays were performed so that no more than 20% of the NAAG was digested, representing the linear range of peptidase activity. Quisqualate (100 μM) was included in parallel assay tubes to confirm the specificity of the measurements.

25

In Vitro Assay of NAALADase Inhibitors on Ischemia

To examine the *in vitro* effect of NAALADase inhibitors on ischemia, cortical cell cultures were treated with compounds of formula I during an ischemic insult (potassium cyanide and 2-deoxyglucose) and for one hour thereafter (for experimental details, see Vornov et al., *J. Neurochem*, Vol. 65, No. 4, pp. 1681-1691 (1995)).

The neuroprotective effect of each tested compound is provided below in TABLE III(a). Neuroprotective effect is expressed as EC_{50} , the concentration which is required to cause a 50% reduction in glutamate toxicity following an ischemic insult.

TABLE III(a)

Compound	EC ₅₀ (nM)
<div data-bbox="212 436 259 468">5</div> <div data-bbox="373 499 1055 934"><p>The chemical structure shows a central phosphate group (P=O, two P-O single bonds) connected to two 3-oxopropyl chains. Each 3-oxopropyl chain is further substituted with a benzyl ester group (-CH₂-C(=O)-O-CH₂-C₆H₅) at the 1-position. The two chains are also substituted with a carboxylate group (-COO-) at the 3-position.</p></div> <div data-bbox="1149 667 1266 699">0.0110</div>	
<div data-bbox="212 1035 259 1066">15</div> <div data-bbox="365 1150 868 1428"><p>The chemical structure shows a central phosphate group (P=O, two P-O single bonds) connected to two 3-oxopropyl chains. Each 3-oxopropyl chain is further substituted with a carboxylate group (-COO-) at the 1-position. The two chains are also substituted with a carboxylate group (-COO-) at the 3-position.</p></div> <div data-bbox="1156 1266 1273 1297">0.0360</div>	
25	

The dose-response of this effect, as measured by the % toxicity at different concentrations of NAALADase inhibitor 2-(phosphonomethyl)pentanedioic acid, is provided below in TABLE III(b) and graphically presented in FIG. 1.

TABLE III(b)

	<u>Dose</u>	<u>% Toxicity</u>
	Control	100.00 \pm 9.0 (n = 5)
10	100 pM	66.57 \pm 4.38 (n = 5)
	1 nM	42.31 \pm 9.34 (n = 5)
	10 nM	33.08 \pm 9.62 (n = 5)
	100 nM	30.23 \pm 9.43 (n = 5)
	1 μ M	8.56 \pm 8.22 (n = 5)

The results show that toxicity decreased as the concentration of 2-(phosphonomethyl)pentanedioic acid increased, suggesting that NAALADase inhibitors would be effective in treating ischemia or neuronal damage caused by ischemia.

The methods for this assay are described in detail below. Specifically, cell cultures were exposed to potassium cyanide and 2-deoxyglucose (2-DG) (10 mM) and analyzed for release of lactate dehydrogenase (LDH).

In Vitro Toxicity of NAAG

To examine the *in vitro* toxicity of NAAG, cortical cell cultures were treated with NAAG (in concentrations ranging from 3 μ M to 3 mM) for 20 minutes. The toxicity measurement for each concentration of NAAG is provided below in TABLE IV and graphically presented in FIG. 2.

TABLE IV

	<u>Dose of NAAG</u>	<u>% Toxicity</u>	
10	3 μ M	3.51	(n = 1)
	10 μ M	4.30 \pm 3.12	(n = 3)
	30 μ M	11.40 \pm 6.17	(n = 3)
	100 μ M	12.66 \pm 5.50	(n = 3)
	300 μ M	13.50 \pm 4.0	(n = 3)
15	1 mM	21.46 \pm 4.20	(n = 3)
	3 mM	45.11 \pm 4.96	(n = 3)

The results show that toxicity increased as the concentration of NAAG increased. The toxicity is attributed to the release of glutamate by NAAG when cleaved by NAALADase.

In Vitro Assay of NAALADase Inhibitors on Toxicity of
NAAG

To examine the effect of NAALADase inhibitors on *in vitro* toxicity of NAAG, cortical cell cultures were

treated with 2-(phosphonomethyl)pentanedioic acid (1 μ M) during exposure to NAAG and for one hour thereafter. The toxicity measurement for each concentration of NAAG is provided below in TABLE V and graphically presented in FIG. 3.

TABLE V

	<u>Dose of NAAG</u>	<u>% Toxicity</u>
	3 μ M	-4.71 (n = 1)
10	10 μ M	-3.08 \pm 0.81 (n = 3)
	30 μ M	-4.81 \pm 1.13 (n = 3)
	100 μ M	-2.87 \pm 0.78 (n = 3)
	300 μ M	-2.09 \pm 0.48 (n = 3)
	1 mM	0.26 \pm 1.11 (n = 3)
15	3 mM	16.83 \pm 8.76 (n = 3)

When compared to the results of FIG.2/TABLE IV, the results of FIG.3/TABLE V show that toxicity decreased considerably after treatment with the NAALADase inhibitor, suggesting that it would be effective in treating glutamate abnormalities.

In Vitro Assay of NAALADASE Inhibitors on Ischemia at Different Times of Administration

To examine the effect of NAALADase inhibitors on in vitro ischemic toxicity at different times of

administration, cortical cell cultures were treated with 2-(phosphonomethyl)pentanedioic acid (i) during an ischemic insult and for one hour thereafter (exposure and recovery); (ii) for one hour following ischemic insult (recovery only); and (iii) for one hour beginning 30 minutes after ischemic insult (delayed 30 minutes). The toxicity measurement for each time of administration is provided below in TABLE VI and graphically presented in FIG. 4.

TABLE VI

<u>Time of Administration</u> <u>Relative to Ischemic Insult</u>	<u>% Toxicity</u>
Control	100.00%
Exposure & Recovery	2.54%
Recovery Only	9.03%
Delayed 30 Minutes	31.49%

The results show that significant neuronal protection is achieved when NAALADase inhibitors are administered during exposure and recovery from an ischemic insult, and even after a 30 minute delay following the ischemic insult.

Protocol for In Vitro Toxicity Assaya. Cell Culture

Dissociated cortical cell cultures are prepared using the papain-dissociation method of Heuttner and Baughman (1986) as modified by Murphy and Baraban (1990). See TABLE VII for the Dissociated Culture Protocol as used herein. Fetuses of embryonic day 17 are removed from timed pregnancy rats (Harlan Sprague Dawley). The cortex is rapidly dissected out in Dulbecco's phosphate-buffered saline, stripped of meninges, and incubated in a papain solution for 15 minutes at 37° C. The tissue is then mechanically triturated and pelleted at 500 g (1000-2000 rpm on swinging bucket Beckman). The pellet is resuspended in a DNAase solution, triturated with a 10 ml pipette x15-20, layered over a "10 x 10" solution containing albumin and trypsin inhibitor (see TABLE VII for an example of a "10 x 10" solution), repelleted, and resuspended in a plating medium containing 10% fetal bovine serum (HyClone A-1111-L), 5% heat-inactivated Equine serum (HyClone A-3311-L), and 84% modified Earle's basal medium (MEM) (Gibco 51200-020) with high glucose (4.5 g/L), and 1 g/L NaHCO₃. Each 24-well plate is pretreated with poly-D-lysine (0.5 ml/well of 10 µg/ml) for 1 h and rinsed with water before plating. Cultures are plated at 2.5 x 10⁶ cells/ml with each well of a 24 well plate receiving 500 µl/well. Alternatively, 35 mm

dishes can be plated at 2 ml/dish, 6 well plates at 2 ml/well, or 12 well plates at 1 ml/well. After plating, 50% of the medium is changed every 3-4 days with growth serum containing 5% heat-inactivated Equine serum (HyClone A-3311-L), 95% modified Earle's basal medium (MEM) (Gibco 51200-020), and 1% L-Glutamine (Gibco 25030-081). Experiments are performed after 21 days in cultures. Cultures are maintained in a 5% CO₂ atmosphere at 37° C. These methodologies are described in further detail below in the TABLE VII.

TABLE VII

DISSOCIATED CULTURE PROTOCOL	
I. PREPARE SOLUTIONS	
Stocks/Solutions	
<p>5 <u>DNAase Stock, 1 ml</u> <u>(100x)</u> 5 mg DNAase I (Worthington LS002004);</p> <p>10 1 ml dissoc. EBSS; freeze as 50 μl aliquots.</p>	<p><u>Dulbecco's PBS, 500 ml</u> 4 gm NaCl (J.T. Baker 3624-01); 1.06 gm Na₂HPO₄·7H₂O (Fisher S373-3); 100 mg KCl (Fisher P217- 500); 100 mg KH₂PO₄ (Sigma P- 0662); 500 ml dH₂O; adjust pH to 7.4 and sterile filter.</p>
<p>15 <u>Dissociated EBSS, 500</u> <u>ml</u> 1.1 gm NaHCO₃; 50 ml EBSS stock (Gibco 14050-025); 450 ml dH₂O; sterile filter.</p>	<p><u>EDTA Stock, 10 ml</u> 184.2 mg EDTA sodium salt (Sigma ED4S); 10 ml dH₂O; sterile filter.</p>

5

10

DISSOCIATED CULTURE PROTOCOL	
<u>10 and 10 Stock, 10 ml</u> 100 mg BSA (Sigma A-4919); 100 mg Trypsin Inhibitor from Egg White (Sigma T-2011); 10 ml dissoc. EBSS; sterile filter.	<u>Poly-D-Lysine Stock, 5 ml</u> 5 mg Poly-D-Lysine, 100-150 K (Sigma P-6407); 5 ml sterile water; keep frozen.
Media	

DISSOCIATED CULTURE PROTOCOL	
	<u>Dissociated growth,</u> <u>500 ml</u> 500 ml MEM (Gibco 51200-020) containing glucose and NaHCO_3 (2.25 gm glucose and 0.5 gm NaHCO_3); 25 ml heat-inactivated Equine Serum (HyClone A-3311-L); 5 ml L-Glutamine (200 mM, 100x stock, Gibco 25030-081); sterile filter. 15 ml heat-inactivated Equine Serum (HyClone A-3311-L); 3 ml L-Glutamine (200 mM, 100x stock, Gibco 25030-081); (Gibco 15140-015); 1 ml Penicillin-Streptomycin stock.
	<u>Plating media, 300 ml</u> 250 ml MEM containing glucose and sodium bicarbonate (2.25 gm glucose and 0.5 gm NaHCO_3 in 500 ml Gibco MEM 51200-020); 30 ml Fetal Bovine Serum (HyClone A-1111-L).

DISSOCIATED CULTURE PROTOCOL

For papain
dissociation:

4 mg Cysteine (C-
8277);
25 ml dissoc. EBSS;
250 μ l Papain stock
(Worthington
LS003126);
place in 37°C
waterbath until
clear.

For DNAase treatment:

DNAase, 5 ml

4.5 ml dissoc. EBSS;
500 μ l "10 and 10" stock;
50 μ l DNAase stock.

"10 and 10", 5 ml

4.5 ml of EBSS;
500 μ l "10 and 10" stock.

II. COAT DISHES

Use poly-d-lysine stock at 1:100 dilution to coat
24-well plates (0.5 ml/well) or at 1:10 dilution
to coat 35 mm glass cover slips (1.0
ml/cover slip).

Leave until end of dissection.

DISSOCIATED CULTURE PROTOCOL

III. DISSECT TISSUE

5 Use Harlan Sprague-Dawley timed pregnancy rats,
ordered to arrive at E-17.

Decapitate, spray abdomen down with 70% EtOH.

Remove uterus through midline incision and place
in sterile dPBS.

10 Remove brains from embryos, leaving them in dPBS.

Brain removal: Penetrate skull and skin with fine
forceps at lambda. Pull back to open posterior
fossa. Then move forceps anteriorly to separate
sagittal suture. Brain can be removed by scooping
15 back from olfactory bulbs under the brain.

Move brains to fresh dPBS; subsequently, dissect
away from cortex.

DISSOCIATED CULTURE PROTOCOL**IV. PAPAIN DISSOCIATION**

____ Transfer cortices equally to two 15 ml tubes containing sterile papain solution, maintained at 37° C.

Triturate x1 with sterile 10 ml pipette.

Incubate only for 15 minutes at 37° C.

Spin at 500 G for 5 minutes (1000-2000 RPM on swinging bucket Beckman).

V. DNAase TREATMENT

____ Remove supernatant and any DNA gel layer from cell pellet (or pick up and remove pellet with pipette).

Move cell pellet to DNAase solution.

Triturate with 10 ml pipette, x15-20.

Layer cell suspension over the "10 and 10" solution by pipetting it against the side of the tubes.

Spin again at 500 G for 5 minutes (cells will spin into "10 and 10" layer).

Wash tube sides with plating media without disturbing pellet.

Pipette off the media wash and repeat the wash.

DISSOCIATED CULTURE PROTOCOL

VI. PLATE

___Add about 4.5 ml plating media to each pellet for 5 ml volume.

5 Re-suspend with 10 ml pipette.

Pool cells into a single tube.

Quickly add 10 μ l of the suspended cells to a hemocytometer so that they do not settle.

10 Count cells per large square, corresponding to 10 million cells/ml.

Put re-suspended cells into a larger container so that they number 2.5 million cells/ml.

Triturate to homogeneity.

15 Finish coating plates:

Aspirate or dump Lysine;

Wash x1 with sterile water and dump.

20 Add plating media, with cells, to the plates as follows:

35 mm dishes	2 ml/dish;
--------------	------------

6 well plate	2 ml/well;
--------------	------------

12 well plate	1 ml/well;
---------------	------------

24 well plate	500 μ l/well.
---------------	-------------------

DISSOCIATED CULTURE PROTOCOL**VII. FEED**

Cultures are usually made on Thursdays.

Start feeding twice a week; beginning the following Monday, feedings on Mondays and Fridays.

Remove 50% of volume and replace with fresh growth media.

b. Ischemic Insult using potassium cyanide and 2-deoxyglucose

Twenty-one to twenty-four days following the initial cortical cell plating, the experiment is performed. The cultures are washed three times in HEPES buffered saline solution containing no phosphate. The cultures are then exposed to potassium cyanide (KCN) (5 mM) and 2-deoxyglucose (2-DG) (10 mM) for 20 minutes at 37° C. These concentrations were shown previously to induce maximal toxicity (Vornov et al., *J. Neurochem*, Vol. 65, No. 4, pp. 1681-1691 (1995)). At the end of 24 hours, the cultures are analyzed for release of the cytosolic enzyme lactate dehydrogenase (LDH), a standard measure of cell lysis. LDH measurements are performed according to the method of Koh and Choi, *J. Neuroscience Methods* (1987).

c. NAAG Induced Neurotoxicity

Cultures are assessed microscopically and those with uniform neuronal densities are used in the NAAG neurotoxicity trials.

5 At the time of the experiment, the cultures are washed once in HEPES-buffered saline solution (HBSS; NaCl 143.4 mM, HEPES 5 mM, KCl 5.4 mM, MgSO₄ 1.2 mM, NaH₂PO₄ 1.2 mM, CaCl₂ 2.0 mM, D-glucose 10 mM) (Vornov et al., 1995) and then exposed to various concentrations of NAAG
10 for 20 minutes at 37° C. NAAG concentrations range from 3 µM to 3 mM, and include 3 µM, 10 µM, 30 µM, 100 µM, 300 µM, 1 mM, and 3 mM. At the end of exposure, the cells are washed once with HEPES buffered saline solution and then replaced with serum free modified Earle's basal
15 medium. The cultures are then returned to the CO₂ incubator for 24 hour recovery.

d. Lactate Dehydrogenase Assay

 Release of the cytosolic enzyme lactate dehydrogenase (LDH), a standard measure of cell lysis, is
20 used to quantify injury (Koh and Choi, 1987). LDH-activity measurements are normalized to control for variability between culture preparations (Koh and Choi, 1987). Each independent experiment contains a control condition in which no NAALADase inhibitors are added; a
25 small amount of LDH activity is found in these controls. This control measurement is subtracted from each

experimental point. These values are normalized within each experiment as a percentage of the injury caused by NAAG/ischemia. Only main effects of NAALADase inhibitors are considered; interactions between dose and condition are not examined statistically.

A measurement of the potency of each compound tested is made by measuring the percentage of LDH release into the growth media after exposure to NAAG/ischemia plus NAALADase inhibitor or NAAG/ischemia plus saline (control). Since high concentrations of glutamate may be toxic to cells in certain circumstances, measurement of glutamate toxicity is observed using LDH as a standard measurement technique.

In Vivo Assay of NAALADase Inhibitors on Cortical Injury following MCAO in SHRSP Rats

To examine the effect of NAALADase inhibitors on cortical injury *in vivo*, the infarct volume was measured in SHRSP rats which had sustained middle cerebral artery occlusion (MCAO) and had subsequently been treated with (i) saline; (ii) 10 mg/kg of 2-(phosphonomethyl)-pentanedioic acid followed by 2 mg/kg/hr of 2-(phosphonomethyl)pentanedioic acid for 1 hour; or (iii) 100 mg/kg of 2-(phosphonomethyl)pentanedioic acid followed by 20 mg/kg/hr of 2-(phosphonomethyl)-pentanedioic acid for one hour.

The cortical injury volume for each group of rats is provided below in TABLE VIII and graphically presented in FIG. 5.

TABLE VIII

5	Cortical Injury Volume (mm ³)±S.E.M.	
	Control	184.62±33.52 (n = 10)
	10 mg/kg	135.00±32.18 (n = 10)
	100 mg/kg	65.23±32.18 (n = 10)
10	Cortical Injury Volume (% injury)±S.E.M.	
	Control	34.44±6.53 (n = 10)
	10 mg/kg ³	29.14±7.68 (n = 10)
	100 mg/kg	13.98±6.64 (n = 10)
15	Cortical Protection (% protection)	
	Control	0%
	10 mg/kg	27%
	100 mg/kg	65%

The results show that cortical injury volume decreased and cortical protection increased as the amount of NAALADase inhibitor increased, further supporting the neuroprotective effect of the NAALADase inhibitor.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Cortical Injury

A colony of SHRSP rats is bred at Johns Hopkins School of Medicine from three pairs of male and female rats obtained from the National Institutes of Health (Laboratory, Sciences Section, Veterinary Resources Program, National Center for Research Resources, Bethesda, MD). All rats are kept in a virus-free environment and maintained on regular diet (NIH 31, Zeigler Bros, Inc.) with water ad libitum. All groups of rats are allowed to eat and drink water until the morning of the experiment.

Transient occlusion of the middle cerebral artery (MCA) is induced by advancing a 4-0 surgical nylon suture into the internal carotid artery (ICA) to block the origin of the MCA (Koizumi, 1986; Longa, 1989; Chen, 1992). The rats are anesthetized with 4% halothane, and maintained with 1.0% to 1.5% halothane in air enriched oxygen using a face mask. Rectal temperature is maintained at $37.0 \pm 0.5^{\circ}\text{C}$ throughout the surgical procedure using a heating lamp. The right femoral artery is cannulated for measuring blood gases (pH, oxygen tension $[\text{PO}_2]$, carbon dioxide tension $[\text{PCO}_2]$) before and during ischemia, for monitoring blood pressure during the surgery. The right common carotid artery (CCA) is exposed through a midline incision; a self-retraining

retractor is positioned between the digastric and mastoid muscles, and the omohyoid muscle is divided. The right external carotid artery (ECA) is dissected and ligated. The occipital artery branch of the ECA is then isolated and coagulated. Next, the right internal carotid artery (ICA) is isolated until the pterygopalatine artery is exposed, and carefully separated from the adjacent vagus nerve. The pterygopalatine artery is ligated with 4-0 silk suture close to its origin.

After the CCA is ligated with 4-0 silk suture, a 4-0 silk suture to prevent bleeding from a puncture site, through which a 2.5 cm length of 4-0 monofilament nylon suture (Ethilon), its tip rounded by heating near a electric cautery, is introduced into the ICA lumen. A 6-0 silk suture is tightened around the intraluminal nylon suture at the bifurcation to prevent bleeding, and the stretched sutures at the CCA and the ICA are released. The nylon suture is then gently advanced as far as 20 mm.

Anesthesia is terminated after 10 minutes of MCA occlusion in both groups, and the rats were awakened 5 minutes thereafter. After 2 hours of ischemia, anesthesia is reanesthetized, and reperfusion is performed by withdrawing the intraluminal nylon suture until the distal tip became visible at the origin of the ICA.

Arterial pH and PaCO₂, and partial pressure of

oxygen (PaO_2) are measured with a self-calibrating Radiometer electrode system (ABL 3; Copenhagen, Denmark). Hemoglobin and arterial oxygen content are measured with a hemoximeter (Radiometer, Model OSM3; Copenhagen, Denmark). Blood glucose is measured with a glucose analyzer (model 2300A, Yellow Springs Instruments, Yellow Springs, OH).

Each group is exposed to 2 hours of right MCA occlusion and 22 hours of reperfusion. All variables but the rectal temperature are measured at baseline, at 15 minutes and 45 minutes of right MCA occlusion. The rectal temperature is measured at baseline, at 0 and 15 min of MCA occlusion, and at 0 and 22 hours of reperfusion.

15

In Vivo Assay of NAALADase Inhibitors on Brain Injury following MCAO in Sprague-Dawley Rats

To examine the neuroprotective effect of NAALADase inhibitors on brain injury *in vivo*, Sprague-Dawley rats were treated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid before and after sustaining a 2 hour transient middle cerebral artery occlusion (MCAO). In the control group ($n = 8$), the rats received an IP injection of saline 30 minutes post-occlusion followed by IV saline infusion at a rate of 0.5 ml/hr. In the drug treated groups, the rats received an IP injection of 2-

(phosphonomethyl)pentanedioic acid at a dose of 100 mg/kg at 20 minutes pre-occlusion (n = 5), 30 minutes post-occlusion (n = 9), 60 minutes post-occlusion (n = 7), or 120 minutes post-occlusion (n = 4), followed by a 20 mg/kg/hr IV infusion for 4 hours (infusion rate = 0.5 ml/hr). There was a 15 minute delay between IP and IV treatments for each rat. Twenty two hours following the reperfusion, the rats were euthanized and their brains were removed. Seven coronal sections (2 mm thick) were taken and stained with 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 20 minutes and then fixed in 10% formalin. The anterior and posterior surface of the most rostral brain section and the posterior surface of each of the other 6 sections were imaged. The quantification of infarct size of each brain was obtained using a computer aided-digital imaging analysis system (LOATS). The brain regions completely lacking TTC-staining were characterized as representative of infarcted tissue. The total infarct volume for each rat was calculated by numeric integration of the respective sequential brain areas.

The total infarct volume for each group of rats is graphically presented in FIG. 6.

Vehicle treated rats exhibited a mean total brain infarct volume of $293 \pm 26 \text{ mm}^3$. Rats treated with 2-(phosphonomethyl)pentanedioic acid either before or after

the ischemic insult exhibited significantly lower mean total brain infarct volumes of $122 \pm 26 \text{ mm}^3$ ($p = 0.003$ vs. vehicle) for 20 minute pre-treatment, $208 \pm 40 \text{ mm}^3$ ($p = 0.2$ vs. vehicle) for 30 minute post-treatment, $125 \pm 57 \text{ mm}^3$ ($p = 0.015$ vs. vehicle) for 60 minute post-treatment, and $133 \pm 35 \text{ mm}^3$ ($p = 0.005$ vs. vehicle) for 120 minute post-treatment. These results indicate that 2-(phosphonomethyl)pentanedioic acid is neuroprotective in rat MCAO model of stroke when administered up to 2 hours post-occlusion.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Brain Injury

Male Sprague-Dawley rats (260-320 g) were used. Prior to the experiment, the rats were individually housed and allowed free access to food and water. Each rat received two surgeries: jugular vein cannulation for IV infusion and MCAO. During surgeries, the rat was anesthetized with 2% halothane delivered in oxygen via an inhalation mask. The body temperature was monitored and regulated at normothermic level using a homeothermic heating system. First, a PE-50 polyethylene catheter was inserted into the right jugular vein. One hour later, the rat was reanesthetized for MCAO surgery. The MCAO was achieved using the endovascular suture method described by Long et al., *Stroke*, Vol. 20, pp. 84-91

(1989). Specifically, the right external carotid artery (ECA) was exposed, coagulated and transected. A 3-0 monofilament nylon suture with a blunted tip was introduced into the proximal stump of the ECA via an arteriotomy and advanced 20 mm from the carotid bifurcation until it lodged in the proximal region of the anterior cerebral artery, thereby occluding the origin of the MCA. The rats were allowed to wake up; 2 hours later, the rats were reanesthetized for reperfusion, during which the nylon suture was retracted to the stump of the ECA allowing blood recirculation to the MCA.

In Vivo Assay of NAALADase Inhibitors on Stroke-Induced Rise in Brain Glutamate Levels

To examine the effect of NAALADase inhibitors on hyperglutamatergic disorders *in vivo*, rats with stroke-induced rise in brain glutamate levels were treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid.

The results are graphically presented in FIGS. 7, 8 and 9.

The results show that 2-(phosphonomethyl)-pentanedioic acid treatment (100 mg/kg IP followed by 20 mg/kg/hr IV) significantly attenuated stroke-induced extracellular glutamate increases in the striatum (FIG. 7) as compared to vehicle treated rats ($p < 0.05$), and completely prevented concurrent glutamate changes in the

parietal cortex ($p < 0.01$; FIG. 8). In contrast, there was no significant effect of the stroke itself on glutamate in the frontal cortex and no subsequent difference between the vehicle and 2-(phosphonomethyl)-pentanedioic acid treated groups (FIG. 9). Values are expressed as % baseline where baseline constitutes the mean of three consecutive 20 minute samples preceding stroke. Absolute basal (pretreatment) values for glutamate (mean \pm SEM) in caudate, parietal and frontal cortices were 0.25 ± 0.1 , 1.1 ± 0.3 and 0.6 ± 0.1 μM , respectively, in the vehicle treated rats, and 0.46 ± 0.1 , 2.0 ± 0.7 and 0.9 ± 0.3 μM , respectively, in the 2-(phosphonomethyl)pentanedioic acid treated rats.

Protocol for In Vivo Assay of NAALADase Inhibitors on Stroke-Induced Rise in Brain Glutamate Levels

Male Sprague Dawley rats (270-330 g, $n = 5-6$ per group) were implanted with concentric microdialysis probes similar to previously described procedures (Britton et al., *J. Neurochem.*, Vol. 67, pp. 324-329 (1996)). In brief, under halothane anaesthesia, probes (constructed in-house using Cuprophane capillary membrane; 10K mw cut off; 2 mm dialyzing length) were implanted into the frontal cortex (AP = +3.5; ML = 3; DIV = 3), caudate nucleus (AP = 0; ML = 3; DV = 6.6), and parietal cortex (AP = -2; ML = 5; DV = 3) (coordinates in

mm relative to bregma and dura, respectively), regions believed to represent core and penumbral areas of ischemia-induced injury. Glutamate levels in dialysate were determined using precolumn o-phthaldialdehyde derivatization, followed by HPLC with fluorometric detection.

Approximately 20 hours after probe implantation, the rats were dialyzed with perfusion fluid (125 mM NaCl, 2.5 mM KCl, 1.18 mM MgCl₂ and 1.26 mM CaCl₂) at a rate of 2.5 µl/min. Following a 60 minute stabilization period, dialysis samples were collected every 20 minutes. After collecting 3 baseline samples, the rats were anaesthetized with halothane and subjected to temporary ischemia using the filament method of MCAO (Britton et al., *Life Sciences*, Vol. 60, No. 20, pp. 1729-1740 (1997)). In brief, the right external carotid artery (ECA) was exposed and its branches coagulated. A 3-0 monofilament nylon suture was introduced into the internal carotid artery via an arteriotomy in the ECA and advanced until it lodged in the proximal region of the anterior cerebral artery, thus occluding the origin of the MCA. The endovascular suture was retracted to allow reperfusion 2 hours after occlusion.

Body temperature was maintained normothermic throughout stroke surgery and reperfusion procedures. The rats were dosed IP with 100 mg/kg 2-

(phosphonomethyl)pentanedioic acid at -20 minute pre-occlusion and IV with 20 mg/kg/hr for 4 hours at the time of occlusion. Dialysis samples were collected every 20 minutes from unanesthetized rats. Following 24 hours of
5 reperfusion, the rats were sacrificed, their brains were removed, and 7 coronal sections (2 mm thick) were taken from the region beginning 1 mm from the frontal pole and ending just rostral to the cortico-cerebellar junction. Analysis of ischemic cerebral damage was achieved using
10 TTC staining and computer assisted image analysis as described by Britton et al. (1997), *supra*.

**In Vivo Assay of NAALADase Inhibitors on Myelin
Formation Following Sciatic Nerve Cryolesion**

15 It was recently demonstrated that NAALADase is down-regulated in glial cells as they start to form myelin and is absent in myelinating Schwann cells. Based on this data, the inventors hypothesized that inhibition of NAALADase may affect the signaling mechanism between
20 axons and Schwann cells and result in increasing myelination. To test this hypothesis, the inventors examined the effect of 2-(phosphonomethyl)pentanedioic acid on nerve regeneration and myelination following cryolesion of the sciatic nerve in male mice.

25 The results are provided below in TABLE IX and graphically presented in FIG. 10(a) and FIG. 10(b).

TABLE IX
IN VIVO EFFECT OF NAALADASE INHIBITORS ON MYELIN
FORMATION FOLLOWING SCIATIC NERVE CRYOLESION

5	2 - (phosphonomethyl) - pentanedioic acid	vehicle
10	ratio of # of myelinated axons (drug/vehicle)	1.5
15	# of myelinated lamellae (ave. + SEM)	16.53 ± 0.65
20	% increase of myelinated lamellae over vehicle	13.77 ± 0.09
20	significance by t-test	p < 0.005

25 As detailed in FIG. 10(a) and FIG. 10(b), both light and transmission electron microscopy (TEM) examination of the nerve 3 mm distal to the site of cryolesion

demonstrated a significant increase in the number of myelinated axons (1.5-fold increase) and myelin thickness (20% increase, $p < 0.005$), as compared to nerves in mice treated with vehicle.

5 FIG. 10(a) and FIG. 10(b) show a photomicrograph of this effect. Sections were stained with toluidine blue which stains myelin. Sciatic nerves treated with 2-(phosphonomethyl)-pentanedioic acid containing implants, compared with sciatic nerves treated with vehicle
10 containing implants, exhibited an increase in myelinated axon number as well as an increase in myelin thickness.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Myelin Formation Following Sciatic Nerve Cryolesion

15 Cryolesion of the mouse sciatic nerve was performed according to Koenig et al., *Science*, Vol. 268, pp. 1500-1503 (June 1995). In brief, each mouse was anesthetized and its sciatic nerve was exposed in the upper thigh and cryolesioned using a copper cryode (diameter = 0.5 mm)
20 that was dipped in liquid nitrogen and repeatedly applied to the upper part of the nerve. The extent of the lesion was approximately 1 mm.

 2-(Phosphonomethyl)pentanedioic acid was incorporated into silicone strips according to the method
25 of Connold et al., *Developmental Brain Res*, Vol. 28, pp. 99-104 (1986), and was implanted at the site of

cryolesion on day 0 and replaced on days 3, 6, 9 and 12. Approximately 2.5 $\mu\text{g/day}$ of 2-(phosphonomethyl)-pentanedioic acid was released from the silicone implants each day. Both right and left sciatic nerves of each mouse were lesioned; right-side nerves were treated with silicone implant strips containing vehicle alone while left-side nerves were treated with silicone implants containing 2-(phosphonomethyl)pentanedioic acid. Fifteen days after surgery, the mice were sacrificed and their sciatic nerve segments were collected and processed for light microscopy and TEM analysis. Randomly chosen fields 2-3 mm distal to the lesion were qualitatively analyzed by light microscopy using 1-micrometer-thick toluidine blue stained cross sections and photographic images were captured.

In Vivo Assay of NAALADase Inhibitors on Parkinson's Disease

To examine the effect of NAALADase inhibitors on Parkinson's Disease *in vivo*, MPTP lesioned mice were treated with 2-(phosphonomethyl)pentanedioic acid or a vehicle.

The percent of dopaminergic neurons for each group of mice is provided below in TABLE X and graphically presented in FIG. 11.

TABLE X
IN VIVO EFFECT OF NAALADASE INHIBITORS ON PARKINSON'S
DISEASE

5	Percent Strial TH Innervation Density (mean \pm SEM)
vehicle/vehicle	24.74 \pm 1.03
10	MPTP/vehicle
	7.82 \pm 0.68
	MPTP/2-(phosphonomethyl)- pentanedioic acid
15	16.28 \pm 0.98

Mice treated with MPTP and vehicle exhibited a substantial loss of functional dopaminergic terminals as compared to non-lesioned mice (approximately 68% loss). Lesioned mice receiving 2-(phosphonomethyl)pentanedioic acid (10 mg/kg) showed a significant recovery of TH-stained dopaminergic neurons ($p < 0.001$). These results indicate that 2-(phosphonomethyl)pentanedioic acid protects against MPTP-toxicity in mice.

25

Protocol for In Vivo Assay of NAALADase Inhibitors on
Parkinson's Disease

MPTP lesioning of dopaminergic neurons in mice was used as an animal model of Parkinson's Disease, as described by Steiner, *Proc. Natl. Acad. Sci.*, Vol. 94, pp. 2019-2024 (March 1997). In brief, four week old male CD1 white mice were dosed IP with 30 mg/kg of MPTP for 5 days. 2-(Phosphonomethyl)pentanedioic acid (10 mg/kg) or a vehicle was administered SC along with the MPTP for 5 days, as well as for an additional 5 days following cessation of MPTP treatment. At 18 days following MPTP treatment, the mice were sacrificed and their brains were removed and sectioned. Immunostaining was performed on sagittal and coronal brain sections using anti-tyrosine hydroxylase (TH) antibodies to quantitate survival and recovery of dopaminergic neurons.

In Vivo Assay of NAALADase Inhibitors on Dynorphin-
Induced Spinal Cord Injury

To examine the neuroprotective effect of NAALADase inhibitors on excitotoxic spinal cord injury in vivo, rats which had sustained dynorphin-induced spinal cord injury were treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid.

The results are graphically presented in FIG. 12. When co-administered with dynorphin A, 2-

(phosphonomethyl)pentanedioic acid (4 μ moles) caused significant improvement in motor scores by 24-hour post-injection, as compared to vehicle treated rats ($p < 0.05$, Kruskal-Wallis comparison). The rats were characterized as ambulatory or not on the basis of their assigned neurological scores (0 to 4). At 24 hours post-injection, 73% of the 15 rats co-treated with 2-(phosphonomethyl)pentanedioic acid were ambulatory, in contrast to 14% of the 14 vehicle co-treated rats ($p < 0.05$). These results indicate that 2-(phosphonomethyl)pentanedioic acid provides effective protection against dynorphin-induced spinal cord injury.

Protocol for In Vivo Assay of NAALADase Inhibitors on

Dynorphin-Induced Spinal Cord Injury

Spinal Subarachnoid Injections

Dynorphin-induced spinal cord injury was performed according to Long et al., *JPET*, Vol. 269, No. 1, pp. 358-366 (1993). In brief, spinal subarachnoid injections were delivered using 30-gauge needles inserted between the L4-L5 vertebrae of male Sprague-Dawley rats (300-350 g). The rats were anesthetized with halothane and dorsal midline incisions were made immediately rostral to the pelvic girdle. By using the vertebral processes as guides, the needle was advanced to pass into the subarachnoid space surrounding the cauda equina. Correct

needle placement was verified by CSF flow from the needle after its insertion. Injections were delivered using a Hamilton microsyringe in a total volume of 20 μ l which contained dynorphin (20 nmol), the cannula flush and 2-
5 (phosphonomethyl)pentanedioic acid or vehicle. After injections, the incisions were treated with the topical antibacterial furazolidone and closed with wound clips. Rapid recovery from the halothane anesthesia enabled neurological evaluations to be made within 5 minutes of
10 injections.

Neurological Evaluations

Neurological function was evaluated using a 5-point ordinal scale, with scores being assigned as follows: 4 = normal motor function; 3 = mild paraparesis, with the
15 ability to support weight and walk with impairment; 2 = paraparesis, with the ability to make walking movements without fully supporting weight; 1 = severe paraparesis, in which rats could make limited hind limb movement, but not walking movement; and 0 = flaccid paralysis, with
20 complete absence of any hind limb movement. Neurological evaluations were made 24 hours after dynorphin A injection.

Statistics

Differences in the neurological scores among
25 treatment groups were determined by means of the Mann-Whitney U test or the Kruskal-Wallis test.

In Vitro Assay of NAALADase Inhibitors on Amyotrophic
Lateral Sclerosis (ALS)

To examine the neuroprotective effect of NAALADase inhibitors on Amyotrophic Lateral Sclerosis (ALS), spinal cord organotypic cultures were treated with threohydroxyaspartate (THA), 2-(phosphonomethyl)-pentanedioic acid, or THA combined with 2-(phosphonomethyl)pentanedioic acid, and assayed for choline acetyltransferase (ChAT) activity.

The ChAT activity for each treatment of the spinal cord organotypic cultures is provided below in TABLE XI and graphically presented in FIG. 13.

TABLE XI
NEUROPROTECTIVE EFFECT OF NAALADASE INHIBITORS IN
SPINAL CORD CULTURE MODEL OF ALS

5	Treatment	ChAT Activity (% of Control)
	control	100 ± 22.1
10	2-(phosphonomethyl)- pentanedioic acid alone	108 ± 18.4
	THA alone	36 ± 12.1
15	2-(phosphonomethyl)- pentanedioic acid and THA	121 ± 18.8

20

As shown in FIG. 13, treatment of the spinal cord organotypic cultures with 100 μ M THA resulted in a reduction of ChAT activity to approximately 36% of control cultures. Co-incubation of the cultures with THA and 2-(phosphonomethyl)pentanedioic acid (100 nM - 10 μ M) significantly protected the cultures from THA toxicity.

25

The dose-response of this effect is provided below in TABLE XII and graphically presented in FIG. 14.

TABLE XII

5 NEUROPROTECTIVE EFFECT OF NAALADASE INHIBITORS IN
 SPINAL CORD CULTURE MODEL OF ALS

		ChAT Activity (% of Control)
10	control	100.0
	THA	0
15	THA and 1 nM 2-(phosphonomethyl)- pentanedioic acid	-23.9 ± 18.6
	THA and 10 nM 2-(phosphonomethyl)- pentanedioic acid	23.1 ± 12.5
20	THA and 100 nM 2-(phosphonomethyl)- pentanedioic acid	87.5 ± 21.7
25	THA and 1 μM 2-(phosphonomethyl)- pentanedioic acid	187.7 ± 32.8

THA and 10 μ M 2-(phosphonomethyl)- 128.7 \pm 17.2
pentanedioic acid

5 Spinal cord cultures were incubated with various
doses of 2-(phosphonomethyl)pentanedioic acid (1 nM to 10
 μ M) in the presence of THA (100 μ M) for 14 days. As
shown in FIG. 14, 2-(phosphonomethyl)pentanedioic acid
exhibited dose-dependent protection against THA-induced
10 toxicity with maximal effects at 1 μ M.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Amyotrophic Lateral Sclerosis (ALS)

Spinal Cord Organotypic Cultures

15 Organotypic cultures were prepared from lumbar
spinal cord of 8 day old rats, as described by Rothstein
et al., *J. Neurochem.*, Vol. 65, No. 2 (1995), and
Rothstein et al., *Proc. Natl. Acad. Sci. USA*, Vol. 90,
pp. 6591-6595 (July 1993). In brief, lumbar spinal cords
20 were removed and sliced into 300 μ M-thick-dorsal-ventral
sections, and five slices were placed on Millipore CM
semipermeable 30-mm-diameter membrane inserts. The
inserts were placed on 1 ml of culture medium in 35-mm-
diameter culture wells. Culture medium consisted of 50%
25 minimal essential medium and phosphate-free HEPES (25
mM), 25% heat-inactivated horse serum, and 25% Hanks'

balanced salt solution (GIBCO) supplemented with D-glucose (25.6 mg/ml) and glutamine (2 mM), at a final pH of 7.2. Antibiotic and antifungal agents were not used. Cultures were incubated at 37° C in 5% CO₂ containing humidified environment (Forma Scientific). Culture medium, along with any added pharmacological agents, was changed twice weekly.

Chronic Toxicity Model with THA

For all experiments, cultures were used 8 days after preparation at which time threohydroxyaspartate (THA; 100 μM), 2-(phosphonomethyl)pentanedioic acid (100 pM - 10 μM), or THA (100 μM) ± 2-(phosphonomethyl)pentanedioic acid (100 pM - 10 μM) were added to the culture medium. Drugs were incubated for an additional 13 to 20 days with the 100 μM THA. At the end of this period, cultures were collected assayed for ChAT activity as described below.

ChAT Assays

To determine choline acetyltransferase (ChAT) activity, the spinal cord tissues in each dish (five slices) were pooled and frozen (-75° C) until assay. ChAT activity was measured radiometrically by described methods using [³H]acetyl-CoA (Amersham; Fonnum, 1975). Protein content of tissue homogenate was determined by a Coomassi Protein Assay kit (Pierce, Rockford, IL).

In Vivo Assay of NAALADase Inhibitors on EthanolConsumption in Alcohol-Preferring Rats

To test the effect of NAALADase inhibitors on ethanol consumption, alcohol-preferring rats were treated with saline or a 50, 100 or 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid prior to ethanol access. The ethanol intake of the rats following treatment is graphically presented in FIG. 15.

As shown in FIG. 15, the 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid exhibited no effect, whereas both the 50 and 100 mg/kg doses significantly reduced ethanol consumption by approximately 25% ($p < 0.05$) during the 1 hour access period. Body weights and 24 hour water intakes were not altered at any of the 3 doses. If 2-(phosphonomethyl)pentanedioic acid is acting centrally, these data suggest that NAALADase may be involved in neuronal systems regulating alcohol-drinking behavior.

20

Saline Baseline: 8.9 ± 0.7

200 mg/kg 2-(phosphonomethyl)pentanedioic acid:

 8 ± 0.5

25

Saline Baseline: 7.8 ± 0.8

100 mg/kg 2-(phosphonomethyl)pentanedioic acid:

 5.8 ± 0.7

Saline Baseline: 8.1 ± 0.6

50 mg/kg 2-(phosphonomethyl)pentanedioic acid:

6.2 ± 0.9

5 Protocol for In Vivo Assay of NAALADase Inhibitors on
 Ethanol Consumption in Alcohol-Preferring Rats

 The effect of systemic administration of 2-(phosphonomethyl)pentanedioic acid was examined on ethanol intake in the alcohol-preferring (P) line of rats, as described by Panocka et al., *Pharm. Biochem. and Behavior*, Vol. 52, No. 2, pp. 255-259 (1995) and Murphy et al., *Alcohol*, Vol. 2, pp. 349-352 (1985). In brief, 2-(phosphonomethyl)pentanedioic acid (50, 100 and 200 mg/kg IP) was tested in female P rats (n = 8) given daily 1 hour scheduled access to a 10% (v/v) ethanol solution. A within-subject design was used where 2-(phosphonomethyl)pentanedioic acid treatments were tested once per week. Baseline ethanol drinking consisted of the mean of the 3 days prior to testing in which saline injections were given. 2-(Phosphonomethyl)pentanedioic acid or saline, administered IP in 1 ml/kg volumes, were injected 10-15 minutes prior to ethanol access. 24 hour water and daily body weights were recorded to assess non-specific drug effects. Results were analyzed using paired t-tests with baseline and test day values serving as the independent variables. Ethanol intake was

recorded as amount of solution consumed (mls).

In Vivo Assay of NAALADase Inhibitors on Nicotine Self-Administration in Male Long-Evans Rats

5 To test the effect of NAALADase inhibitors on nicotine self-administration, male Long-Evans rats trained to self-administer nicotine were treated with a 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid prior to nicotine access. The cumulative nicotine intake
10 of the rats following treatment is graphically presented in FIG. 16.

 The results show that the 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid reduced nicotine self-administration from 23 to 5 infusions during the 1 hour
15 access period. As graphically presented in FIG. 17, the cumulative food intake of the rats also decreased during the same period of time. While these data suggest that factors other than 2-(phosphonomethyl)pentanedioic acid may be responsible for the reduction in nicotine self-
20 administration, they do not disprove NAALADase's involvement in the neuronal systems regulating nicotine use. The effect on the rats' food intake could be attributed to toxicity caused by an excessive drug dose.

25

Protocol for In Vivo Assay of NAALADase Inhibitors on
Nicotine Self-Administration in Male Long-Evans Rats

Male Long-Evans rats were trained to self-administer nicotine on a fixed ratio schedule of reinforcement, as described by Corrigall et al., *Psychopharmacology*, Vol. 104, No. 2, pp. 171-176 (1991) and Corrigall et al., *Psychopharmacology*, Vol. 107, Nos. 2-3, pp. 285-289 (1992). In brief, male Long-Evans rats were food deprived for a short period of time (24-48 hours) and trained to press a lever in an operant responding chamber on an FR-1 schedule of food reinforcement. Once trained, each rat was surgically prepared with a chronic intravenous catheter implanted into the jugular vein. The rats were allowed 1 week to recover from surgery.

After 1 week, nicotine self-administration studies were initiated on an FR-1 with a 60 second signaled time-out following each infusion. During time-out, responding on the lever had no scheduled consequence. Nicotine self-administration sessions were 60 minutes in duration. Each nicotine infusion contained 30 μ g of nicotine/kg rat and were delivered in a volume of 54 μ l over an infusion duration of 0.3 seconds. 15 minutes before the self-administration sessions, the rats were pre-treated intraperitoneally with 2-(phosphonomethyl)-pentanedioic acid at doses of 10, 20 and 30 mg/kg. Food intake was monitored during the nicotine self-administration

sessions to assess non-specific drug effects.

In Vitro Assay of NAALADase Inhibitors
on Cancer

5 To examine the effect of NAALADase inhibitors on
cancer cell line, LNCAP cells (a prostate cancer cell
line) were treated with quisqualate acid (in
concentrations ranging from 10 nM to 1 μ M) and 2-
(phosphonomethyl)pentanedioic acid (in concentrations
10 ranging from 100 pM to 10 nM). The 3H-thymidine
measurement for each concentration of quisqualate acid
and 2-(phosphonomethyl)pentanedioic acid is provided in
TABLE XIII below and graphically represented in FIG. 18
and FIG. 19, respectively.

TABLE XIII

<u>3H-Thymidine Incorporation (dpm/well)</u>			
	<u>Dose</u>	<u>Quisqualic Acid</u>	<u>2-(phosphonomethyl)- pentanedioic acid</u>
20	Control	4813 \pm 572	4299 \pm 887
	10 pM	--	3078 \pm 1006
25	100 pM	--	2062 \pm 595
	1 nM	3668 \pm 866	1001 \pm 52
	10 nM	2137 \pm 764	664 \pm 366
30	100 nM	1543 \pm 312	--
	1 μ M	1295 \pm 181	--

The results show that LNCAP cell proliferation (as measured by the incorporation of 3H-thymidine) decreased significantly as the concentration of the NAALADase inhibitors increased, suggesting that the compounds of the present invention would be effective in treating cancer, particularly prostate cancer.

Protocol for In Vitro Cancer Assay

Cells in RPMI 1640 medium containing 10% Fetal Calf Serum (FCS) are plated in 24 well plates and allowed to adhere for 24 hours before addition of quisqualic acid (10^{-9} to 10^{-6}) or 2-(phosphonomethyl)pentanedioic acid (10^{-11} to 10^{-8}) for 7 days. On the 7th day, the cells are pulsed with 3H-thymidine for 4 hours, harvested and measured for radioactivity. Values represent means +/- SEM of 6 separate cell wells for each treatment. All experiments are performed at least twice.

To control for non-specific cytostatic effects of quisqualate acid and 2-(phosphonomethyl)pentanedioic acid, the agents are simultaneously evaluated on a non-NAALADase containing prostate cell line, DU145 (Carter et al., Proc. Natl. Acad. Sci. USA, (93) 749-753, 1996). If the treatments with quisqualate acid and 2-(phosphonomethyl)pentanedioic have no significant effect on cell growth, the NAALADase inhibiting activity of the agents are uniquely responsible for their cytostatic

effects on prostate carcinoma cell lines.

Cell Lines and Tissue Culture

LNCAP cells are obtained from Dr. William Nelson at the Johns Hopkins School of Medicine in Baltimore, MD. DU145 cells are obtained from American Type Culture Collection (Rockville, MD). Cells are grown in RPMI-1640 media supplemented with 5% heat-inactivated fetal calf serum, 2 mM-glutamine, 100 units/ml penicillin, and 100 μ g/ml streptomycin (Paragon) in a humidified incubator at 37°C in a 5% CO₂/95% air atmosphere.

[3H] Thymidine Incorporation Assays

The cells are suspended at 1×10^3 cells/ml in RPMI-1640 media and seeded into 24-well plates at 500 μ l per well. After 24 hour incubation, various concentrations of quisqualic acid (Sigma) or the potent NAALADase inhibitor 2-(phosphonomethyl)pentanedioic acid (synthesized according to the methods of Jackson et al., *J. Med. Chem.*, Vol. 39, No. 2, pp. 619-622, is added to the wells and the plates are returned to the incubator. On days 3, 5 and 7, media and drug are refreshed. On the 8th day following seeding, each well is pulsed with 1 μ Ci ³H-thymidine (New England Nuclear) for 4 hours. Media is then removed and the wells washed 2 times with phosphate buffered saline (pH=7.4). The contents of each well is subsequently solubilized 250 μ l of 0.2 N NaOH and transferred to scintillation vials. 5 ml UltimaGold

(Packard) scintillation cocktail is added and radioactivity is quantitated using a Beckman LS6001 scintillation counter.

5 The purity and/or identity of all synthetic compounds is ascertained by thin layer chromatography, High Pressure Liquid Chromatography (HPLC), mass spectrometry, and elemental analysis. Proton Nuclear Magnetic Resonance (NMR) spectra are obtained using a Bruker spectrometer. Chemical shifts are reported in
10 parts per million relative to tetramethylsilane as internal standard. Analytical thin-layer chromatography (TLC) is conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE). Visualization of the plates is accomplished by using UV light, phosphomolybdic acid-ethanol, and/or iodoplatinate charring. Flash
15 chromatography is conducted on Kieselgel 60, 230-400 mesh (E. Merck, Darmstadt, West Germany). Solvents are either reagent or HPLC grade. Reactions are run at ambient temperature and under a nitrogen atmosphere unless
20 otherwise noted. Solutions are evaporated under reduced pressure on a Buchi rotary evaporator.

EXAMPLES

25 The following examples are illustrative of the present invention and are not intended to be limitations thereon. Unless otherwise indicated, all percentages are

based upon 100% by weight of the final composition.

EXAMPLE 1

Synthesis of 2-[[[2-(carboxy)propyl]hydroxy- phosphinyl]methyl]pentanedioic acid

Di-tert-butyl 2-methylenepentanedioate

Tert-butyl acrylate (465 g, 3.628 mol) was warmed to 100° C under nitrogen, then hexamethylphosphorous triamide (10 g, 61.2 mmol) was added dropwise and the addition rate was adjusted to maintain the reaction temperature at 100° C. The reaction mixture was allowed to cool, then poured over a plug of silica (~1000 ml) and washed completely off the silica with 4:1 hexane/ethyl acetate. The solvent was removed under reduced pressure and the resulting oil was distilled. Some material was collected from room temperature to 50° C under high vacuum, and discarded. The temperature was then raised to -80° C and the product (300 g, 65 %, b.p. 67-70° C at 300 μ) was collected as a clear oil. ¹H NMR (CDCl₃): δ 1.4 (m, 18H), 2.4 (t, 2H), 2.6 (t, 2H), 5.5 (s, 1H), 6.0 (s, 1H).

Di-tert-butyl 2-[(hydroxyphosphinyl)methyl]pentanedioate

A mixture of ammonium phosphinate (162.6 g, 1.96 mol) and 1,1,1,3,3,3-hexamethyldisilazane (316 g, 1.96 mol) was heated to 105° C for 2 hours. The reaction mixture was cooled in an ice bath and di-tert-butyl 2-

methylenepentane-1,5-dioate (251 g, 0.979 mol) dissolved in dichloromethane (1000 ml) was added dropwise. The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was then quenched with
5 distilled water (500 ml) and the organic layer was retained. The aqueous layer was washed a second time with dichloromethane and the combined organic layers were dried over magnesium sulfate. Then the solvent was removed under reduced pressure leaving a slightly yellow
10 oil (315 g, 100 %). This product was found to be of sufficient purity for use in the next reaction. ¹H NMR (CDCl₃): δ 1.4 (m, 18H), 1.9 (m, 3H), 2.1 (m, 1H), 2.3 (m, 2H), 2.7 (m, 1H), 6.5 & 7.9 (d, 1H, the P-H), 11.0 (s, 1H).

15 Di-tert-butyl 2-[(tert-butoxyphosphinyl)methyl]-pentanedioate

To a solution of di-tert-butyl 2-[(hydroxyphosphinyl)methyl]pentane-1,5-dioate (315 g, 0.977 mol) in dichloromethane (1000 ml) cooled in an ice bath and
20 under nitrogen were added tert-butanol (123.1 g, 1.66 mol), 4-dimethylaminopyridine (1 g, 8.2 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (281 g, 1.47 mol). The reaction was allowed to stir overnight. Water was added to the reaction mixture and the dichloromethane
25 layer was retained and dried, and the solvent was removed under reduced pressure. The resulting residue was

purified by column chromatography and the desired product was eluted with 1:1 to 2:3 hexane/ethyl acetate. The fractions containing product were concentrated under reduced pressure leaving a clear oil (260 g, 70 %). ¹H NMR (CDCl₃): δ 1.4 (m, 27H), 1.8 (m, 1H), 1.9 (m, 2H), 2.1 (m, 1H), 2.3 (m, 2H), 2.7-2.8 (m, 1H), 6.7 & 8.0 (d, 1H, the P-H).

Di-tert-butyl 2-[[[2-(benzylcarboxy)propyl]tert-butoxyphosphinyl]methyl]pentanedioate

To a solution of di-tert-butyl 2-[(tert-butoxyphosphinyl)methyl]pentane-1,5-dioate (13.62 g, 36.0 mmol) and benzyl methacrylate (6.35 g, 36.0 mmol) in THF (100 ml) under nitrogen was added sodium hydride (0.14 g, 60 % dispersion in oil, 3.60 mmol). After three hours, the reaction mixture was poured into water (300 ml) and ether (100 ml) was added. The organic layer was separated and retained, and the aqueous layer was washed again with ether (100 ml). The combined organic extracts were dried over magnesium sulfate and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography and the product was eluted with 2:3 EtOAc/Hexane. The solvent was removed under reduced pressure leaving a clear oil (10.5 g, 53 %). ¹H NMR (CDCl₃): δ 1.3 (m, 3H), 1.5 (m, 27H), 1.7 (m, 2H), 1.9 (m, 2H), 2.2 (m, 4H), 2.6 (m, 1H), 2.9 (m, 1H), 5.1 (m, 2H), 7.3 (m, 5H).

2-[[[2-(Benzylcarboxy)propyl]hydroxyphosphinyl]-
methyl]pentanedioic acid

To a solution of di-tert-butyl 2-[[[2-(benzyl-
carboxy)propyl]tert-butoxyphosphinyl]methyl]pentane-1,5-
5 dioate (1.6 g, 2.89 mmol) in dichloromethane (10 ml)
under nitrogen was added trifluoroacetic acid (10 ml).
The reaction mixture was stirred for two hours and then
concentrated under reduced pressure. Additional
dichloromethane was added to the reaction residue and
10 removed under reduced pressure. The product was
dissolved in ethyl acetate and washed with water, then
the organic layer was dried over magnesium sulfate and
the solvent was removed under reduced pressure leaving a
clear oil (800 mg, 72 %). ¹H NMR (D₂O): δ 1.2 (m, 3H),
15 1.6-1.8 (m, 4H), 2.1 (m, 2H), 2.2 (m, 2H), 2.6 (m, 1H),
2.8 (m, 1H), 5.0 (m, 2H), 7.3 (m, 5H). Analysis
calculated for C₁₇H₂₃PO₈ · 1.0 H₂O: C, 50.50; H, 6.23.
Found: C, 50.52; H, 5.92.

Di-tert-butyl 2-[[[2-(carboxy)propyl]tert-butoxy-
20 phosphinyl]methyl]pentanedioate

A solution of di-tert-butyl 2-[[[2-(benzyl-
carboxy)propyl]tert-butoxyphosphinyl]methyl]pentane-1,5-
dioate (8.9 g, 16.1 mmol), palladium on carbon catalyst
(10 %, 1.0 g) and ethyl acetate (100 ml) was shaken under
25 hydrogen (60 psi) for 16 hours. The reaction mixture was
filtered over celite and the filtrate was concentrated

under reduced pressure leaving a clear oil (7.5 g, 100 %). ^1H NMR (CDCl_3): δ 1.3 (d, 3H), 1.4-1.5 (m, 27H), 1.8 (m, 2H), 1.9 (m, 2H), 2.2 (m, 4H), 2.7 (m, 1H), 2.9 (m, 1H).

5 2-[[[2-(Carboxy)propyl]hydroxyphosphinyl]methyl]-pentanedioic acid

To a solution of di-tert-butyl 2-[[[2-(carboxy)-propyl]tert-butoxyphosphinyl]methyl]pentane-1,5-dioate (2.1 g, 4.53 mmol) in dichloromethane (10 ml) under
10 nitrogen was added trifluoroacetic acid (10 ml). The reaction mixture was stirred for two hours and then concentrated under reduced pressure. Additional dichloromethane was added to the reaction residue and removed under reduced pressure. The resulting residue
15 was triturated with acetonitrile, then dried under reduced pressure leaving a thick clear oil (1.2 g, 89 %). ^1H NMR (D_2O): δ 1.2 (d, 3H), 1.9 (m, 4H), 2.2 (m, 2H), 2.4 (m, 2H), 2.8 (m, 2H). Analysis calculated for $\text{C}_{10}\text{H}_{17}\text{PO}_8 \cdot 0.2 \text{ CH}_3\text{CN}$: C, 41.03; H, 5.83. Found: C, 41.05;
20 H, 5.92.

EXAMPLE 2

A patient is at risk of injury from an ischemic event. The patient may be pretreated with an effective
25 amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the

pretreatment, the patient would be protected from any injury due to the ischemic event.

EXAMPLE 3

5 A patient is suffering from an ischemic event. The patient may be administered during or after the event, an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would recover or
10 would not suffer any significant injury due to the ischemic event.

EXAMPLE 4

15 A patient has suffered injury from an ischemic event. The patient may be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would recover from the injury due to the ischemic event.

20

EXAMPLE 5

 A patient is suffering from a glutamate abnormality. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the
25 present invention. It is expected that after the treatment, the patient would be protected from further

injury due to the glutamate abnormality or would recover from the glutamate abnormality.

EXAMPLE 6

5 A patient is suffering from or has suffered from a nervous insult, such as that arising from a neurodegenerative disease or a neurodegenerative process. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the
10 present invention. It is expected that after the treatment, the patient would be protected from further injury due to the nervous insult or would recover from the nervous insult.

EXAMPLE 7

15 A patient is suffering from Parkinson's disease. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the
20 treatment, the patient would be protected from further neurodegeneration or would recover from Parkinson's disease.

EXAMPLE 8

25 A patient is suffering from ALS. The patient may then be administered an effective amount of a compound or

a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from ALS.

5

EXAMPLE 9

A patient is suffering from epilepsy. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from epilepsy.

10

EXAMPLE 10

A patient is suffering from abnormalities in myelination/demyelination processes. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from the abnormalities in myelination/demyelination processes.

15

20

EXAMPLE 11

A patient is suffering from or has suffered from a cerebrovascular accident, such as stroke. The patient

25

may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any injury due to the cerebrovascular accident.

EXAMPLE 12

A patient is suffering from a head trauma. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury resulting from the head trauma.

EXAMPLE 13

A patient is suffering from a spinal trauma. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic injury resulting from the spinal trauma.

EXAMPLE 14

A patient is about to undergo surgery. The patient may be administered an effective amount of a compound or

a pharmaceutical composition of the present invention.
It is expected that after the treatment, the patient
would not develop any ischemic brain, spinal or
peripheral injury resulting from or associated with the
5 surgery.

EXAMPLE 15

A patient is suffering from focal ischemia, such as
that associated with thromboembolytic occlusion of a
10 cerebral vessel, traumatic head injury, edema or brain
tumors. The patient may then be administered an
effective amount of a compound or a pharmaceutical
composition of the present invention. It is expected
that after the treatment, the patient would be protected
15 from or would recover from any brain, spinal or
peripheral injury resulting from the focal ischemia.

EXAMPLE 16

A patient is suffering from global ischemia. The
20 patient may then be administered an effective amount of
a compound or a pharmaceutical composition of the present
invention. It is expected that after the treatment, the
patient would be protected from or would recover from any
brain, spinal or peripheral injury resulting from the
25 global ischemia.

EXAMPLE 17

A patient is suffering from a cardiac arrest. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury associated with the cardiac arrest.

EXAMPLE 18

A patient is suffering from hypoxia, asphyxia or perinatal asphyxia. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury associated with the hypoxia, asphyxia or perinatal asphyxia.

EXAMPLE 19

A patient is suffering from a cerebro-cortical injury. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain injury

resulting from the cerebro-cortical injury.

EXAMPLE 20

5 The patient is suffering from an injury to the
caudate nucleus. The patient may then be administered an
effective amount of a compound or a pharmaceutical
composition of the present invention. It is expected
that after the treatment, the patient would be protected
from or would recover from any ischemic brain injury
10 resulting from the injury to the caudate nucleus.

EXAMPLE 21

15 A patient is suffering from a cortical injury due to
a condition identified in these examples. The patient
may then be administered an effective amount of a
compound or a pharmaceutical composition of the present
invention. It is expected that after the treatment, the
patient would be protected from further injury, or would
exhibit at least 65% to at least 80% recovery from the
20 cortical injury.

EXAMPLE 22

25 A patient is suffering from multiple sclerosis. The
patient may then be administered an effective amount of
a compound or a pharmaceutical composition of the present
invention. It is expected that after the treatment, the

patient would be protected from further demyelination or would recover from multiple sclerosis.

EXAMPLE 23

5 A patient is suffering from a peripheral neuropathy caused by Guillain-Barré syndrome. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would
10 be protected from further demyelination or would recover from the peripheral neuropathy.

EXAMPLE 24

 The patient is suffering from alcoholism. The
15 patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for alcohol would be suppressed.

EXAMPLE 25

20 A patient is suffering from nicotine dependence. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the
25 treatment, the patient's craving for nicotine would be suppressed.

EXAMPLE 26

The patient is suffering from cocaine dependence. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for cocaine would be suppressed.

EXAMPLE 27

A patient is suffering from heroine dependence. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for heroine would be suppressed.

EXAMPLE 28

The patient is suffering from compulsive overeating, obesity or severe obesity. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's compulsion to eat would be suppressed.

EXAMPLE 29

A patient is suffering from pathological gambling. The patient may then be administered an effective amount

of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's compulsion to gamble would be suppressed.

5

EXAMPLE 30

The patient is suffering from ADD. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's symptoms of inattention, impulsivity and/or hyperactivity would be suppressed.

10

EXAMPLE 31

A patient is suffering from Tourette's syndrome. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's simple, complex, respiratory and vocal tics would be suppressed.

15

20

EXAMPLE 32

A patient is suffering from adenocarcinoma of the prostate. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. After this initial

25

treatment, the patient may optionally be administered the same or a different compound of the present invention in intermittent or continuous doses by subdural pump. It is expected that the treatment(s) would prevent recurrences
5 of the adenocarcinoma, or inhibit (i.e., arrest development of) or relieve (i.e., cause regression of) the adenocarcinoma tumor cells.

EXAMPLE 33

10 A patient is suffering from adenocarcinoma of the prostate. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention by direct injection into the tumor. After this initial treatment, the
15 patient may optionally be administered an effective amount of the same or a different compound of the present invention in intermittent or continuous doses by implantation of a biocompatible polymeric matrix delivery system. It is expected that the treatment(s) would
20 prevent recurrences of the adenocarcinoma, or inhibit (i.e., arrest development of) or relieve (i.e., cause regression of) the adenocarcinoma tumor cells.

EXAMPLE 34

25 A patient is diagnosed with benign prostatic hyperplasia. The patient may then be administered an

effective amount of a compound or a pharmaceutical composition of the present invention by direct injection into the tumor. After this initial treatment, the patient may optionally be administered the same or a different compound of the present invention in intermittent or continuous doses by injection, subdural pump or polymeric matrix implant. It is expected that after the treatment(s), the benign prostatic hyperplastic cells would not develop into carcinoma.

10

EXAMPLE 35

A patient is suffering from adenocarcinoma of the prostate. The adenocarcinoma does not appear to have metastasized. The patient undergoes surgery to remove the adenocarcinoma. After post-surgical recovery, the patient may be locally administered an effective amount of a compound or a pharmaceutical composition of the present invention in intermittent or continuous doses by injection, subdural pump or polymeric matrix implant. It is expected that after the treatment, the patient would be protected from recurrences of the adenocarcinoma, and any residual tumorous cells would be inhibited (i.e., arrested in development) or relieved (i.e., caused to regress).

25

EXAMPLE 36

A patient is suffering from metastatic adenocarcinoma of the prostate. Although the adenocarcinoma appears to have metastasized, the patient nevertheless undergoes surgery to remove the adenocarcinoma. The patient may then be locally administered an effective amount of a compound or a pharmaceutical composition of the present invention approximately from the time of initial diagnosis through post-surgical recovery. After post-surgical recovery, the patient may continue the same treatment by a regimen of periodic local administration, and carefully monitored for adverse side-effects. It is expected that after the treatments, the patient would be protected from recurrences of the adenocarcinoma, and any residual tumorous cells would be inhibited (i.e., arrested in development) or relieved (i.e., caused to regress).

EXAMPLE 37

A patient is suffering from cancer as defined herein. An effective amount of a compound or a pharmaceutical composition of the present invention may be administered directly to the cancer cells. After this initial treatment, the patient may be optionally administered an effective amount of the same or a different compound of the present invention by direct

injection, subdural pump or implantation of a biocompatible polymeric matrix delivery system. It is expected that after the treatment(s), the patient would be protected from recurrences of the cancer, and the
5 cancer would be inhibited (i.e., arrested in development) or relieved (i.e., caused to regress).

EXAMPLE 38

A patient is diagnosed with a disease, disorder or
10 condition as identified in these examples. An effective amount of a compound or a pharmaceutical composition of the present invention may then be administered to the patient intravenously, intramuscularly, intraventricularly to the brain, rectally,
15 subcutaneously, intranasally, through a catheter with or without a pump, orally, through a transdermal patch, topically, or through a polymer implant. After the treatment, the patient's condition would be expected to improve.

20

EXAMPLE 39

A patient is diagnosed with a disease, disorder or condition as identified in these examples. A compound or a pharmaceutical composition of the present invention may
25 then be administered to the patient in the form of a 100 mg/kg bolus, optionally followed by a 20 mg/kg per hour

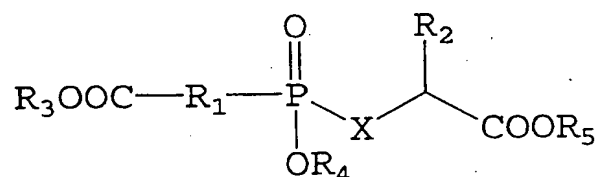
intravenous infusion over a two-hour period. After the treatment, the patient's condition would be expected to improve.

5 The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the
10 scope of the following claims.

WE CLAIM:

1. A compound of formula I:

5



I

10 or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

15 R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain
20 alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

25 R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇

cycloalkenyl and Ar, wherein said R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_9 alkoxy, C_2 - C_9 alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, and amino.

20

2. The compound of claim 1, wherein X is CH_2 .

3. The compound of claim 2, wherein:

R_2 is $-(CH_2)_2COOR_9$; and

R_9 is selected from the group consisting of hydrogen, C_1 - C_9 straight or branched chain alkyl, C_2 - C_9

straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of
5 carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

10

4. The compound of claim 3, wherein R₃, R₄, R₅, and R₉ are hydrogen.

5. The compound of claim 3, which is selected from
15 the group consisting of:

2-[[2-carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic acid;

20 2-[[2-carboxypentyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

25 2-[[2-carboxy-3-naphthylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]-

pentanedioic acid;

2-[[2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]-
5 methyl]pentanedioic acid;

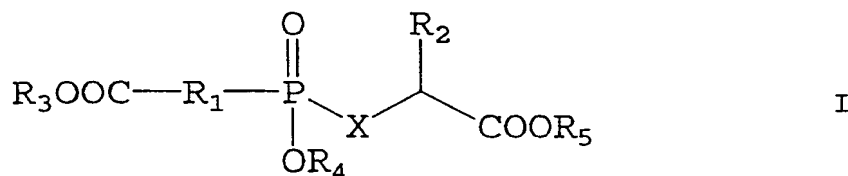
2-[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxyphosphinyl]methyl]pentanedioic acid; and

10 pharmaceutically acceptable salts, hydrates and prodrugs thereof.

6. The compound of claim 5, which is 2-[[2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid,
15 or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

7. A method of treating a glutamate abnormality in an animal, comprising administering to said animal an
20 effective amount of a compound of formula I:



25

or a pharmaceutically acceptable salt, hydrate or prodrug

thereof, wherein:

X is CR_6R_7 , O or NR_8 ;

5 R_1 is selected from the group consisting of $\text{C}_1\text{-C}_9$ straight or branched chain alkyl, $\text{C}_2\text{-C}_9$ straight or branched chain alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl and Ar, wherein said R_1 is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl, halo, hydroxy, 10 nitro, trifluoromethyl, $\text{C}_1\text{-C}_6$ straight or branched chain alkyl, $\text{C}_2\text{-C}_6$ straight or branched chain alkenyl, $\text{C}_1\text{-C}_9$ alkoxy, $\text{C}_2\text{-C}_9$ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

15 R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently selected from the group consisting of hydrogen, $\text{C}_1\text{-C}_9$ straight or branched chain alkyl, $\text{C}_2\text{-C}_9$ straight or branched chain alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl and Ar, wherein said R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently unsubstituted or substituted 20 with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, $\text{C}_1\text{-C}_6$ straight or branched chain alkyl, $\text{C}_2\text{-C}_6$ straight or branched chain alkenyl, $\text{C}_1\text{-C}_9$ alkoxy, $\text{C}_2\text{-C}_9$ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and 25

Ar is selected from the group consisting of 1-

naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

8. The method of claim 7, wherein the compound is administered in combination with at least one additional therapeutic agent.

9. The method of claim 7, wherein the glutamate abnormality is selected from the group consisting of epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease, schizophrenia, chronic pain, ischemia, peripheral neuropathy, traumatic brain injury and physical damage to the spinal cord.

10. The method of claim 9, wherein the glutamate abnormality is ischemia.

11. The method of claim 9, wherein the glutamate abnormality is stroke.

12. The method of claim 9, wherein the glutamate
5 abnormality is Parkinson's Disease.

13. The method of claim 9, wherein the glutamate abnormality is Amyotrophic Lateral Sclerosis (ALS).

10 14. The method of claim 9, wherein the glutamate abnormality is ischemic spinal cord injury.

15. The method of claim 7, wherein X is CH₂.

15 16. The method of claim 15, wherein:

R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy,
20
25

benzyloxy, amino, and Ar.

17. The method of claim 16, wherein R_3 , R_4 , R_5 , and R_9 are hydrogen.

5

18. The method of claim 16, wherein the compound is selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

10 2-[[[(2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl)methyl]pentanedioic acid;

15 2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

20 2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

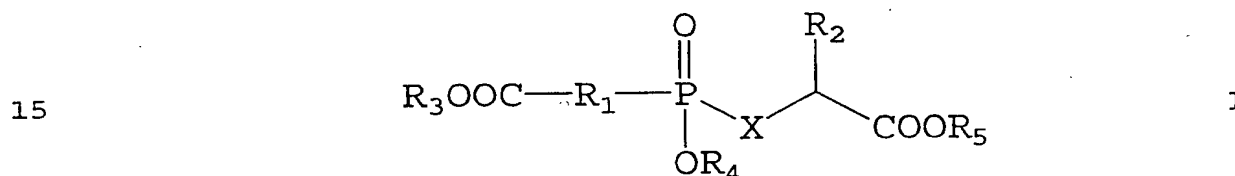
25 2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-

phosphinyl)methyl]pentanedioic acid; and
pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

19. The method of claim 18, wherein the compound is 2-[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid, or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

10 20. A method of effecting a neuronal activity in an
animal, comprising administering to said animal an
effective amount of a compound of formula I:



or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

20 X is CR_6R_7 , O or NR_8 ;

R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl,

C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain

alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

21. The method of claim 20, wherein the compound is administered in combination with at least one additional therapeutic agent.

22. The method of claim 20, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration, and treatment of a neurological disorder.

23. The method of claim 22, wherein the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke associated with brain damage, demyelinating disease and neurological disorder relating to neurodegeneration.

24. The method of claim 23, wherein the peripheral neuropathy is caused by Guillain-Barré syndrome.

25. The method of claim 23, wherein the demyelinating disease is multiple sclerosis.

26. The method of claim 23, wherein the neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, and amyotrophic lateral sclerosis.

27. The method of claim 20, wherein X is CH₂.

28. The method of claim 27, wherein:

10 R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

29. The method of claim 28, wherein R₃, R₄, R₅, and R₉ are hydrogen.

30. The method of claim 28, wherein the compound is

selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;

5 2-[[[(2-carboxybutyl)hydroxyphosphinyl]methyl]pentanedioic
acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;

2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl]methyl]-
pentanedioic acid;

10 2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]-
methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl]methyl]-
pentanedioic acid;

15 2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl]methyl]pentanedioic acid;

2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl]methyl]pentanedioic acid;

2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxy-
phosphinyl]methyl]pentanedioic acid;

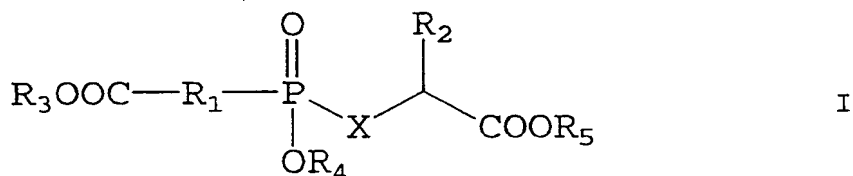
20 2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-
phosphinyl]methyl]pentanedioic acid; and

pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

25 31. The method of claim 30, wherein the compound is
2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-

dioic acid, or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

32. A method of treating a compulsive disorder, comprising administering to a patient in need thereof an effective amount of a compound of formula I:



10

or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

15 R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently
 20 selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and
 25 Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently

selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

33. The method of claim 32, wherein the compound is administered in combination with at least one additional therapeutic agent.

34. The method of claim 32, wherein the compulsive disorder is selected from the group consisting of drug dependence, eating disorders, pathological gambling, attention deficit disorder (ADD) and Tourette's syndrome.

5

35. The method of claim 34, wherein the drug dependence is alcohol dependence.

36. The method of claim 34, wherein the drug
10 dependence is nicotine dependence.

37. The method of claim 30, wherein X is CH_2 .

38. The method of claim 37, wherein:

15 R_2 is $-(\text{CH}_2)_2\text{COOR}_9$; and

R_9 is selected from the group consisting of hydrogen, $\text{C}_1\text{-C}_9$ straight or branched chain alkyl, $\text{C}_2\text{-C}_9$ straight or branched chain alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl and Ar, wherein said R_9 is unsubstituted
20 or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, $\text{C}_1\text{-C}_6$ straight or branched chain alkyl, $\text{C}_2\text{-C}_6$ straight or branched chain
25 alkenyl, $\text{C}_1\text{-C}_9$ alkoxy, $\text{C}_2\text{-C}_9$ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

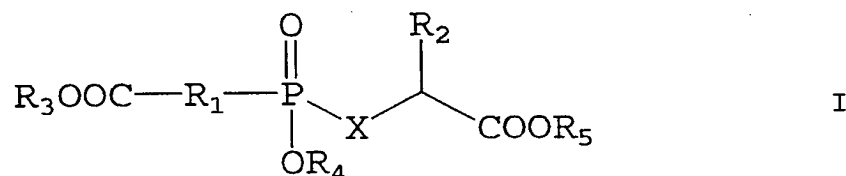
39. The method of claim 38, wherein R_3 , R_4 , R_5 , and R_9 are hydrogen.

40. The method of claim 38, wherein the compound is
5 selected from the group consisting of:
2-[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[(2-carboxybutyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
10 2-[[(2-carboxypentyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl]methyl]-
pentanedioic acid;
2-[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]-
15 methyl]pentanedioic acid;
2-[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl]methyl]-
pentanedioic acid;
2-[[(2-benzyloxycarbonyl)-3-phenylpropyl]hydroxy-
phosphinyl]methyl]pentanedioic acid;
20 2-[[(2-methoxycarbonyl)-3-phenylpropyl]hydroxy-
phosphinyl]methyl]pentanedioic acid;
2-[[(3-carboxy-2-methoxycarbonyl)propyl]hydroxy-
phosphinyl]methyl]pentanedioic acid;
2-[[(4-carboxy-2-methoxycarbonyl)butyl]hydroxy-
25 phosphinyl]methyl]pentanedioic acid; and
pharmaceutically acceptable salts, hydrates and prodrugs

thereof.

41. The method of claim 40, wherein the compound is
2-[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-
5 dioic acid, or a pharmaceutically acceptable salt,
hydrate or prodrug thereof.

42. A method of treating a prostate disease in an
animal, comprising administering to said animal an
10 effective amount of a compound of formula I:



15

or a pharmaceutically acceptable salt, hydrate or prodrug
thereof, wherein:

X is CR₆R₇, O or NR₈;

R₁ is selected from the group consisting of C₁-C₉
20 straight or branched chain alkyl, C₂-C₉ straight or
branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl and Ar, wherein said R₁ is unsubstituted or
substituted with one or more substituent(s) independently
selected from the group consisting of carboxy, carbonyl,
25 C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy,
nitro, trifluoromethyl, C₁-C₆ straight or branched chain

alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

5 R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted
10 with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and
15

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or
20 substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.
25

43. The method of claim 42, wherein the compound is administered in combination with at least one additional therapeutic agent.

5 44. The method of claim 42, wherein said prostate disease is prostate cancer or benign prostatic hyperplasia.

45. The method of claim 42, wherein X is CH₂.

10

46. The method of claim 43, wherein:

R₂ is - (CH₂)₂COOR₉; and

R₉ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

15

20

25 47. The method of claim 46, wherein R₃, R₄, R₅, and R₉ are hydrogen.

48. The method of claim 46, wherein the compound is selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

5 2-[[[(2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl)methyl]pentanedioic acid;

10 2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

15 2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

20 2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxyphosphinyl)methyl]pentanedioic acid; and

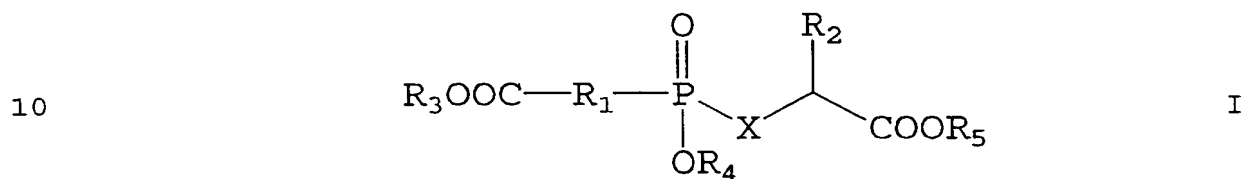
pharmaceutically acceptable salts, hydrates and prodrugs thereof.

25

49. The method of claim 48, wherein the compound is

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentane-
dioic acid, or a pharmaceutically acceptable salt,
hydrate or prodrug thereof.

5 50. A method of inhibiting NAALADase enzyme
activity in an animal, comprising administering to said
animal an effective amount of a compound of formula I:



or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

15 X is CR_6R_7 , O or NR_8 ;

R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently selected from the group consisting of hydrogen, C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl and Ar, wherein said R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_9 alkoxy, C_2 - C_9 alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, and amino.

51. The method of claim 50, wherein the compound is administered in combination with at least one additional

therapeutic agent.

52. The method of claim 50, wherein X is CH₂.

5 53. The method of claim 52, wherein:

R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of
hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉
straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-
10 C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted
or substituted with one or more substituent(s)
independently selected from the group consisting of
carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl,
halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or
15 branched chain alkyl, C₂-C₆ straight or branched chain
alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy,
benzyloxy, amino, and Ar.

20 54. The method of claim 53, wherein R₃, R₄, R₅, and
R₉ are hydrogen.

55. The method of claim 53, wherein the compound is
selected from the group consisting of:

25 2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;

2-[[[(2-carboxybutyl)hydroxyphosphinyl]methyl]pentanedioic

acid;

2-[[(2-carboxypentyl)hydroxyphosphinyl)methyl]pentane-
dioic acid;

2-[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]-
5 pentanedioic acid;

2-[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]-
methyl]pentanedioic acid;

2-[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]-
pentanedioic acid;

10 2-[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;

2-[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;

15 2-[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;

2-[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-
phosphinyl)methyl]pentanedioic acid; and

pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

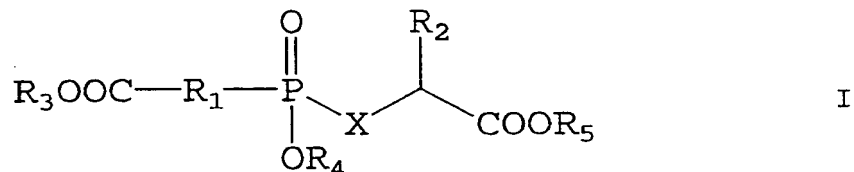
20

56. The method of claim 55, wherein the compound is
2-[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentane-
dioic acid, or a pharmaceutically acceptable salt,
hydrate or prodrug thereof.

25

57. A pharmaceutical composition comprising:

(i) an effective amount of a compound of formula I:



5

or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

10 R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently
15 selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and
20 Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇,
25 and R₈ are independently unsubstituted or substituted

with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino; and

(ii) a pharmaceutically acceptable carrier.

58. The pharmaceutical composition of claim 57, wherein X is CH₂.

59. The pharmaceutical composition of claim 58, wherein:

R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of

hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s)
5 independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy,
10 benzyloxy, amino, and Ar.

60. The pharmaceutical composition of claim 59, wherein R₃, R₄, R₅, and R₉ are hydrogen.

15 61. The pharmaceutical composition of claim 59, wherein the compound is selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid;
20 2-[[[(2-carboxybutyl)hydroxyphosphinyl]methyl]pentanedioic acid;
2-[[[(2-carboxypentyl)hydroxyphosphinyl]methyl]pentanedioic acid;
2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl]methyl]-
25 pentanedioic acid;
2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]-

methyll]pentanedioic acid;
2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl]methyll]-
pentanedioic acid;
2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxy-
5 phosphinyl]methyll]pentanedioic acid;
2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl]methyll]pentanedioic acid;
2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxy-
phosphinyl]methyll]pentanedioic acid;
10 2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-
phosphinyl]methyll]pentanedioic acid; and
pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

15 62. The pharmaceutical composition of claim 61,
wherein the compound is 2-[[[(2-carboxypropyl)hydroxy-
phosphinyl]methyll]pentanedioic acid, or a
pharmaceutically acceptable salt, hydrate or prodrug
thereof.

20 63. The pharmaceutical composition of claim 57,
wherein the amount of the compound of formula I is
effective for inhibiting NAALADase enzyme activity in an
animal.

25

64. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for treating a glutamate abnormality in an animal.

5

65. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for effecting a neuronal activity in an animal.

10

66. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for treating a compulsive disorder in an animal.

15

67. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for treating a prostate disease in an animal.

AMENDED CLAIMS

[received by the International Bureau on 15 June 1999 (15.06.99);
original claims 1 and 50 amended; remaining claims unchanged (2 pages)]

cycloalkenyl and Ar, wherein said R_2 , R_3 , R_4 , R_5 , R_6 , R_7 ,
and R_8 are independently unsubstituted or substituted
with one or more substituent(s) independently selected
from the group consisting of carboxy, carbonyl, C_3 - C_6
5 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro,
trifluoromethyl, C_1 - C_6 straight or branched chain
alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6
alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, amino,
and Ar; provided that when X is $-\text{CH}_2-$, R_1 is $-(\text{CH}_2)_2-$,
10 R_2 is $-(\text{CH}_2)_2\text{CO}_2\text{CH}_2\text{C}_6\text{H}_5$, R_3 is benzyl, and R_4 is hydrogen,
then R_5 is not benzyl; and when X is $-\text{CH}_2-$, R_1 is $-(\text{CH}_2)_2-$,
 R_2 is $-(\text{CH}_2)_2\text{CO}_2\text{H}$, R_3 is hydrogen, and R_4 is
hydrogen, then R_5 is not hydrogen; and

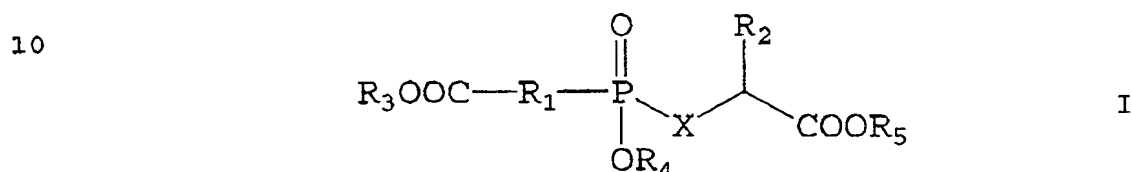
Ar is selected from the group consisting of 1-
15 naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl,
2-furyl, 3-furyl, tetrahydrofuranyl,
tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-
pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar
is unsubstituted or substituted with one or more
20 substituent(s) independently selected from the group
consisting of carboxy, carbonyl, halo, hydroxy, nitro,
trifluoromethyl, C_1 - C_6 straight or branched chain
alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6
alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, and
25 amino.

2. The compound of claim 1, wherein X is CH_2 .

3. The compound of claim 2, wherein:

2-[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-dioic acid, or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

5 50. A method of inhibiting NAALADase enzyme activity in an animal, comprising administering to said animal an effective amount of a compound of formula I:



or a pharmaceutically acceptable salt, hydrate or
15 prodrug thereof, wherein:

X is CR_6R_7 , O or NR_8 ;

R_1 is selected from the group consisting of C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl and Ar, wherein said R_1 is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_9 alkoxy, C_2 - C_9 alkenyloxy, phenoxy, benzyloxy, amino, and Ar; provided that when X is $-\text{CH}_2-$, R_1 is $-(\text{CH}_2)_2-$, R_2 is $-(\text{CH}_2)_2\text{CO}_2\text{H}$, R_3 is hydrogen, and R_4 is hydrogen, then R_5 is not hydrogen.

Compound 3 is Neuroprotective in a
Cell Culture Model of Stroke

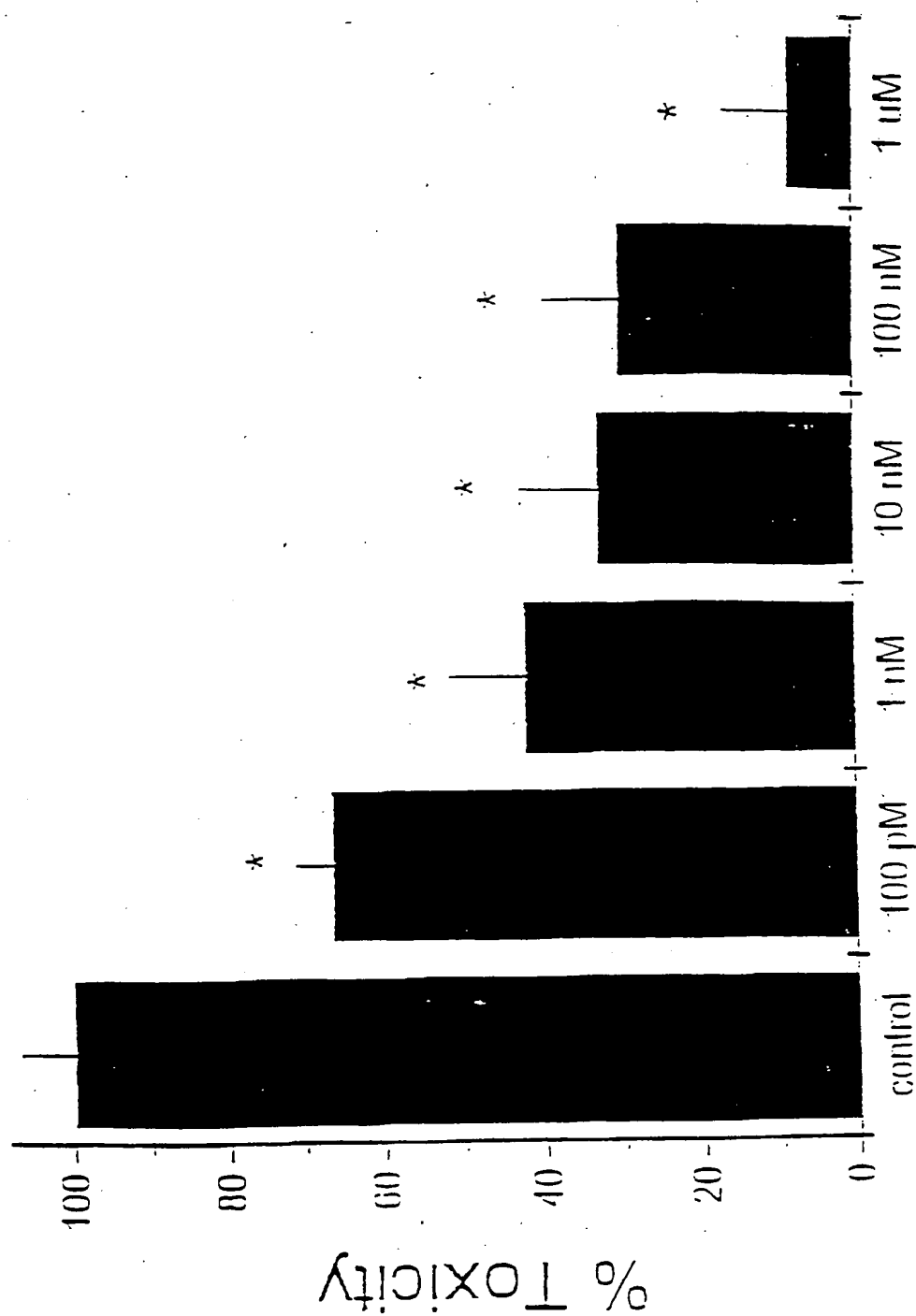


FIGURE 1 Dose of Compound 3

NAAAG Toxicity

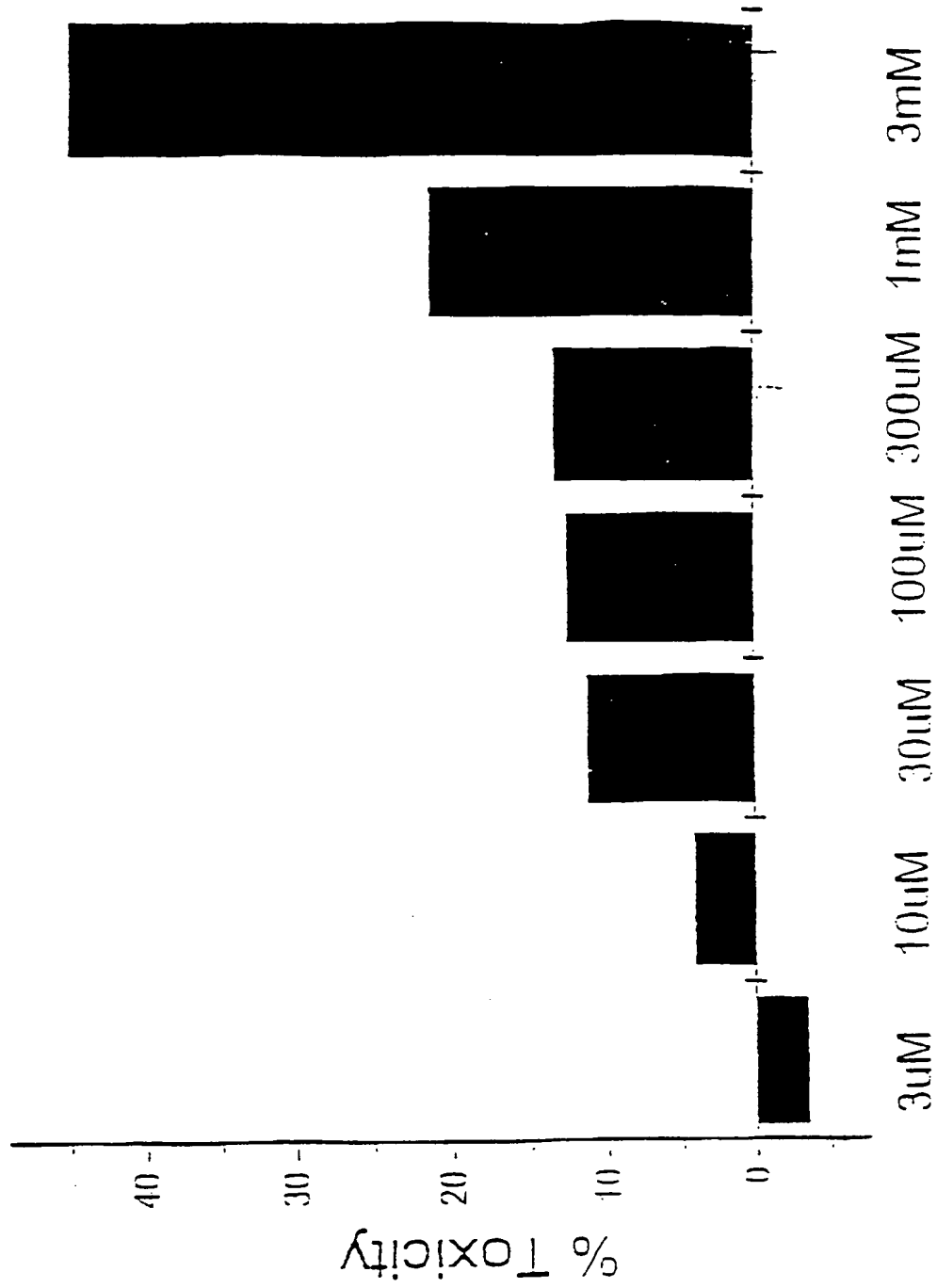


FIGURE 2 Dose of NAAAG

NAAG TOXICITY BLOCKED BY COMPOUND 3

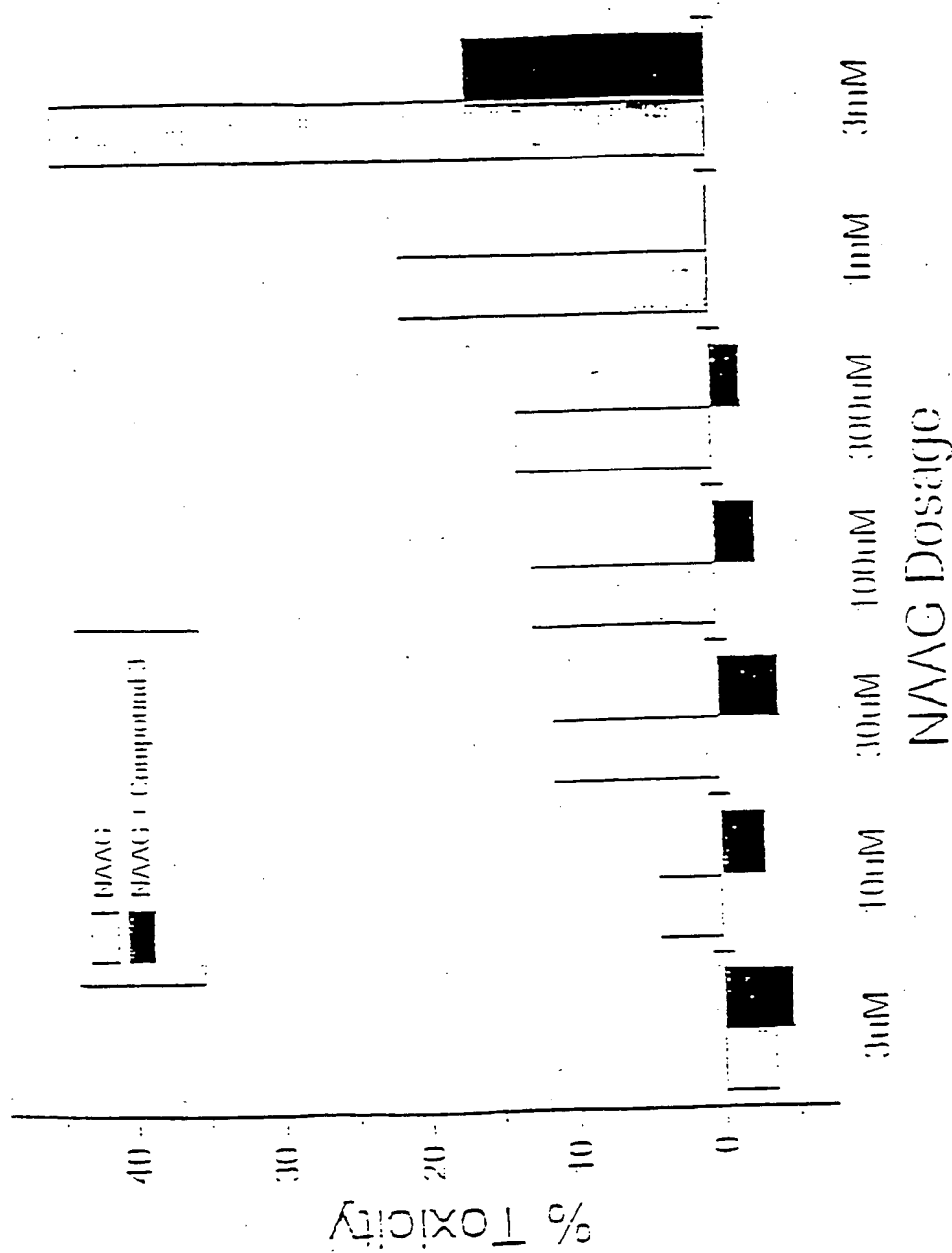


FIGURE 3

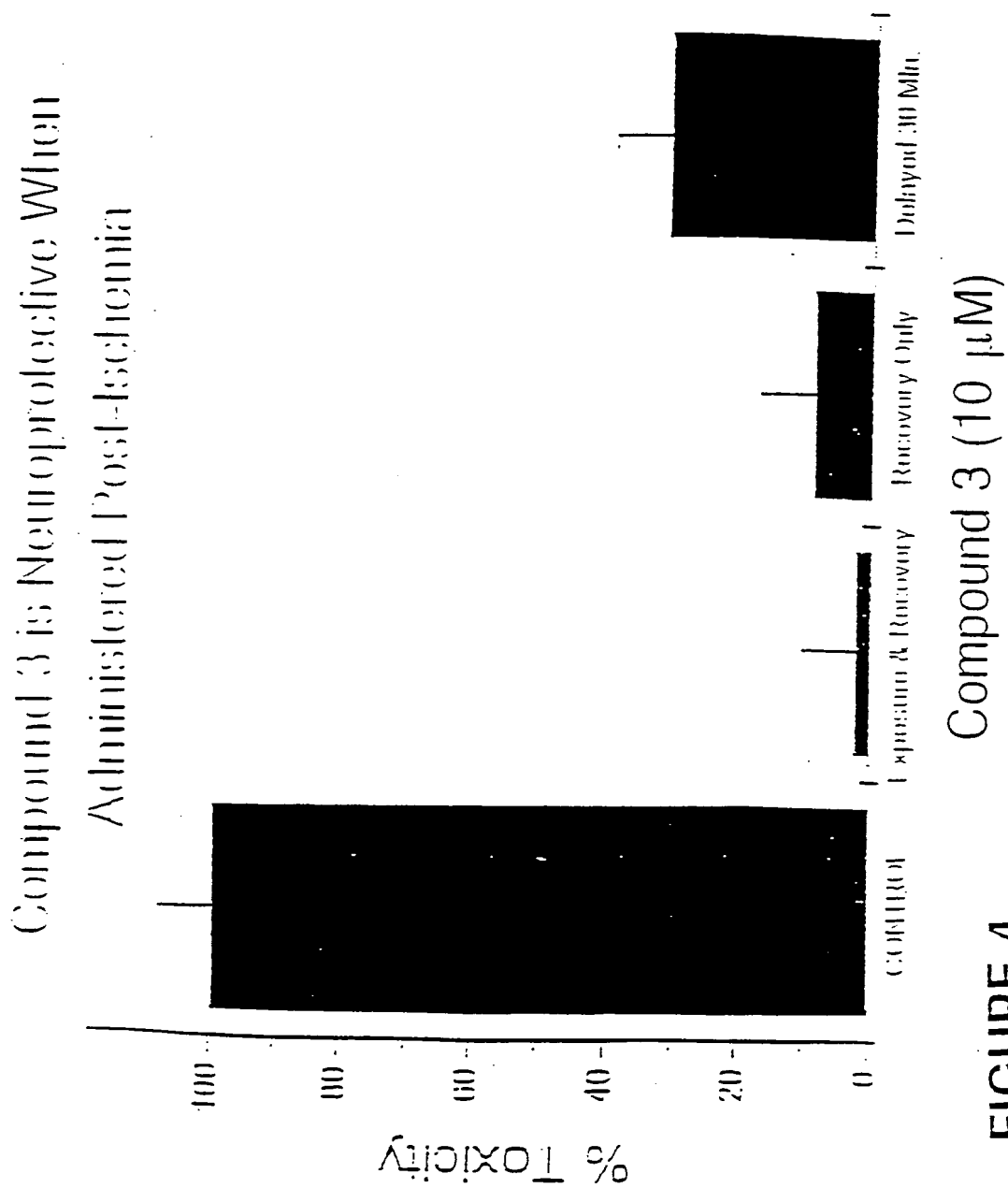


FIGURE 4

Compound 3 is Neuroprotective
in Rat MCAO Model of Stroke

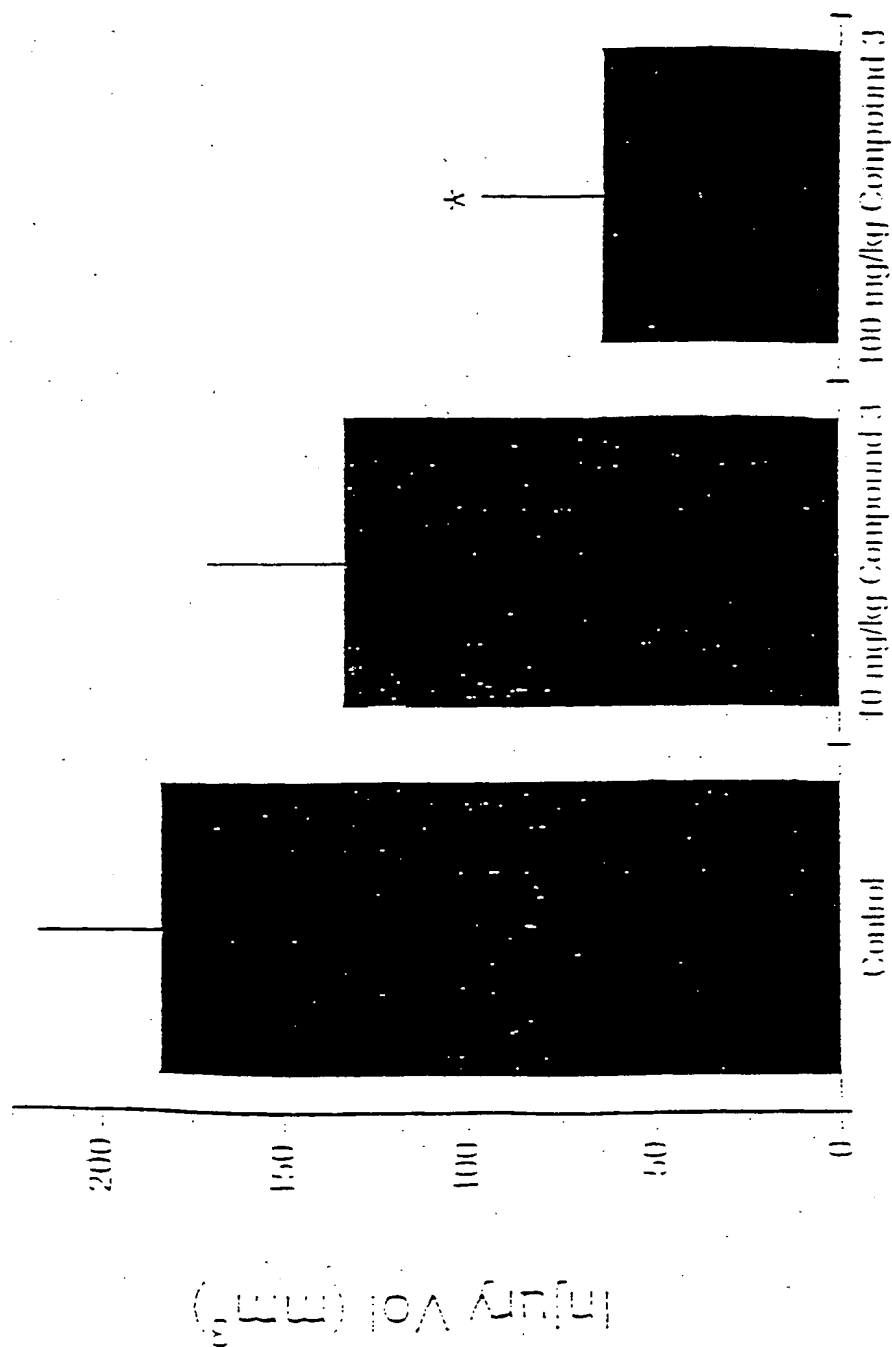


FIGURE 5

COMPOUND 3 IS NEUROPROTECTIVE IN RAT MCAO
MODEL OF STROKE WHEN ADMINISTERED UP
TO 2 HOURS POST-OCCLUSION

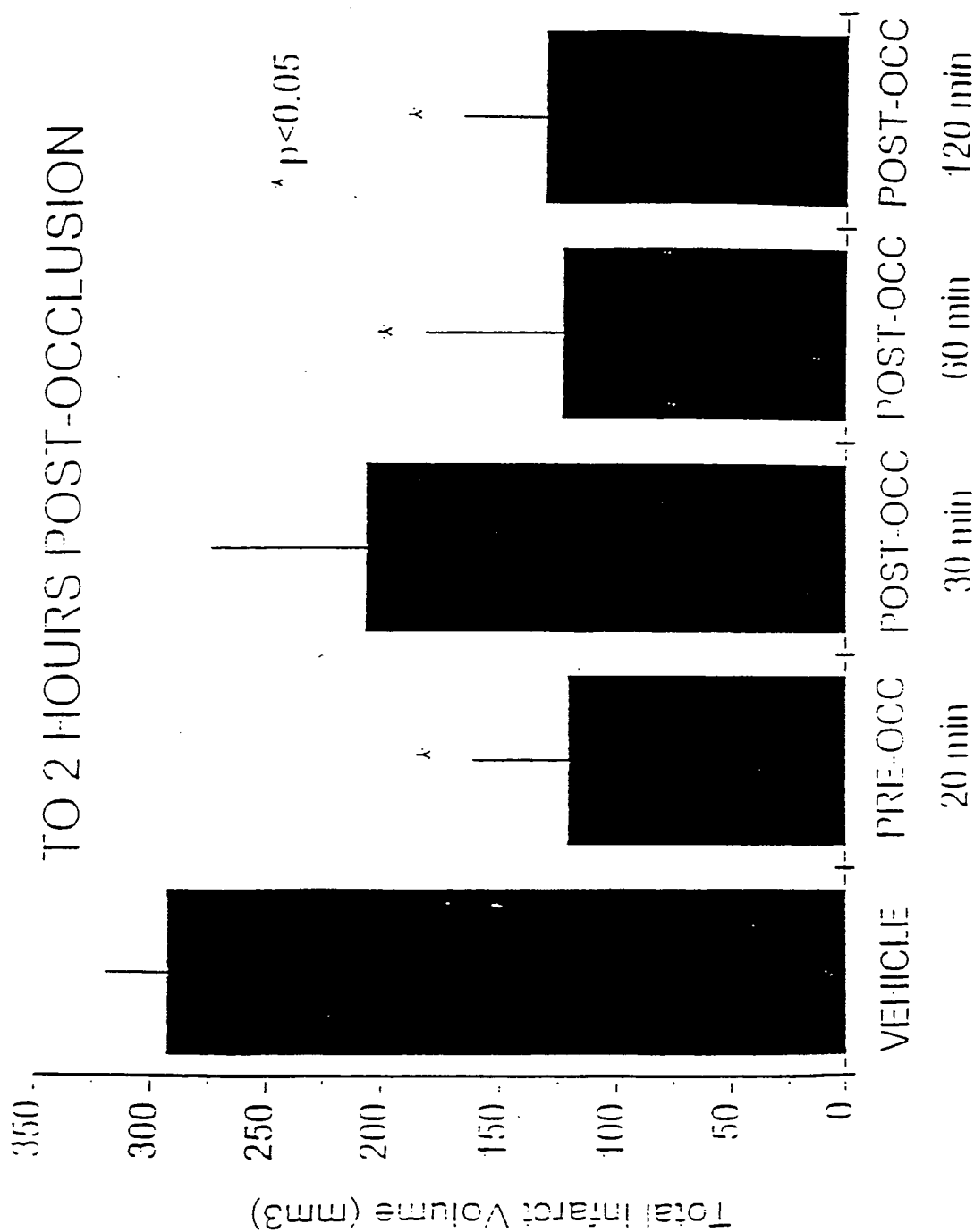


FIGURE 6

STRIATUM

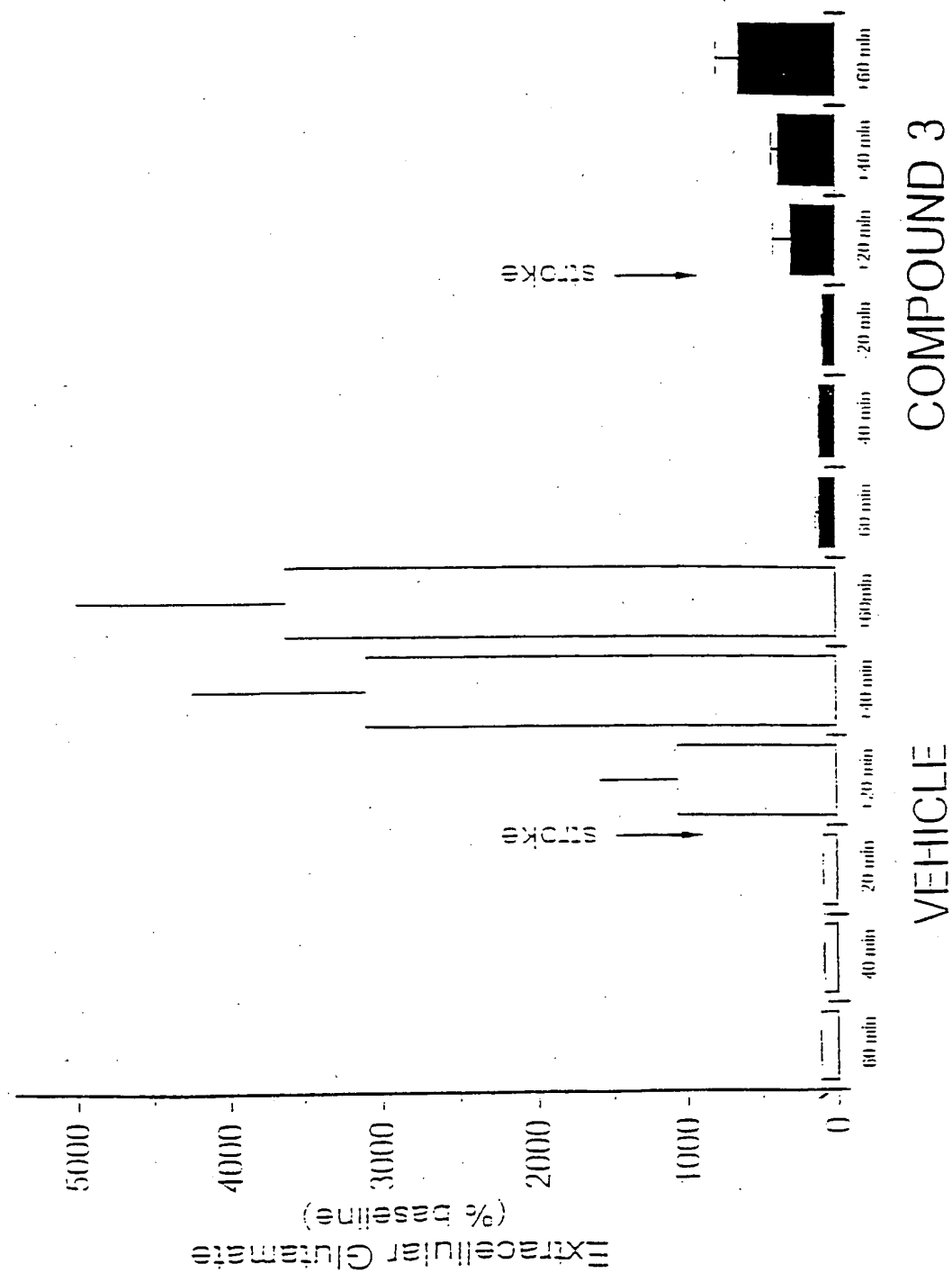
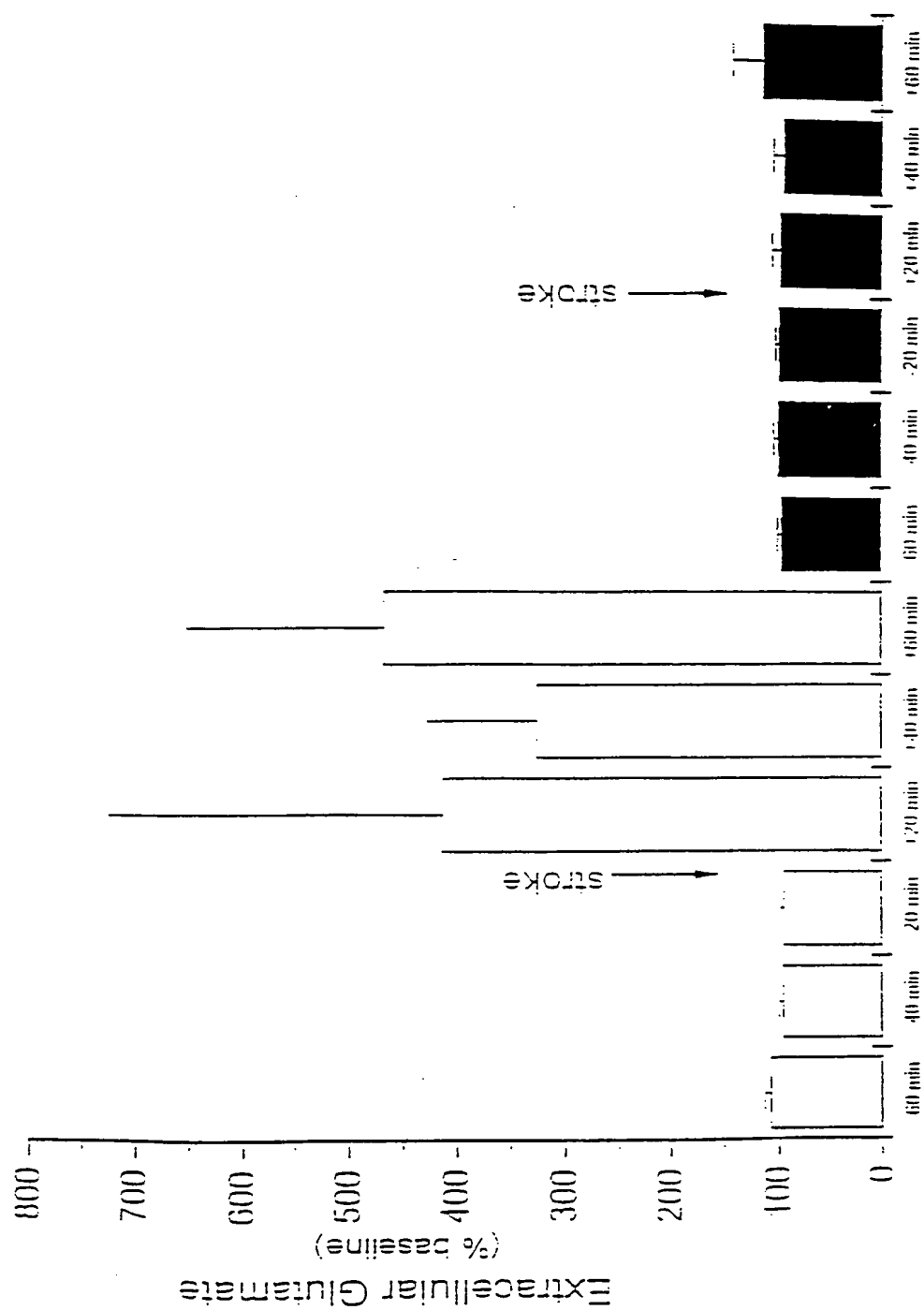


FIGURE 7

PARIETAL CORTEX



COMPOUND 3

VEHICLE

FIGURE 8

FRONTAL CORTEX

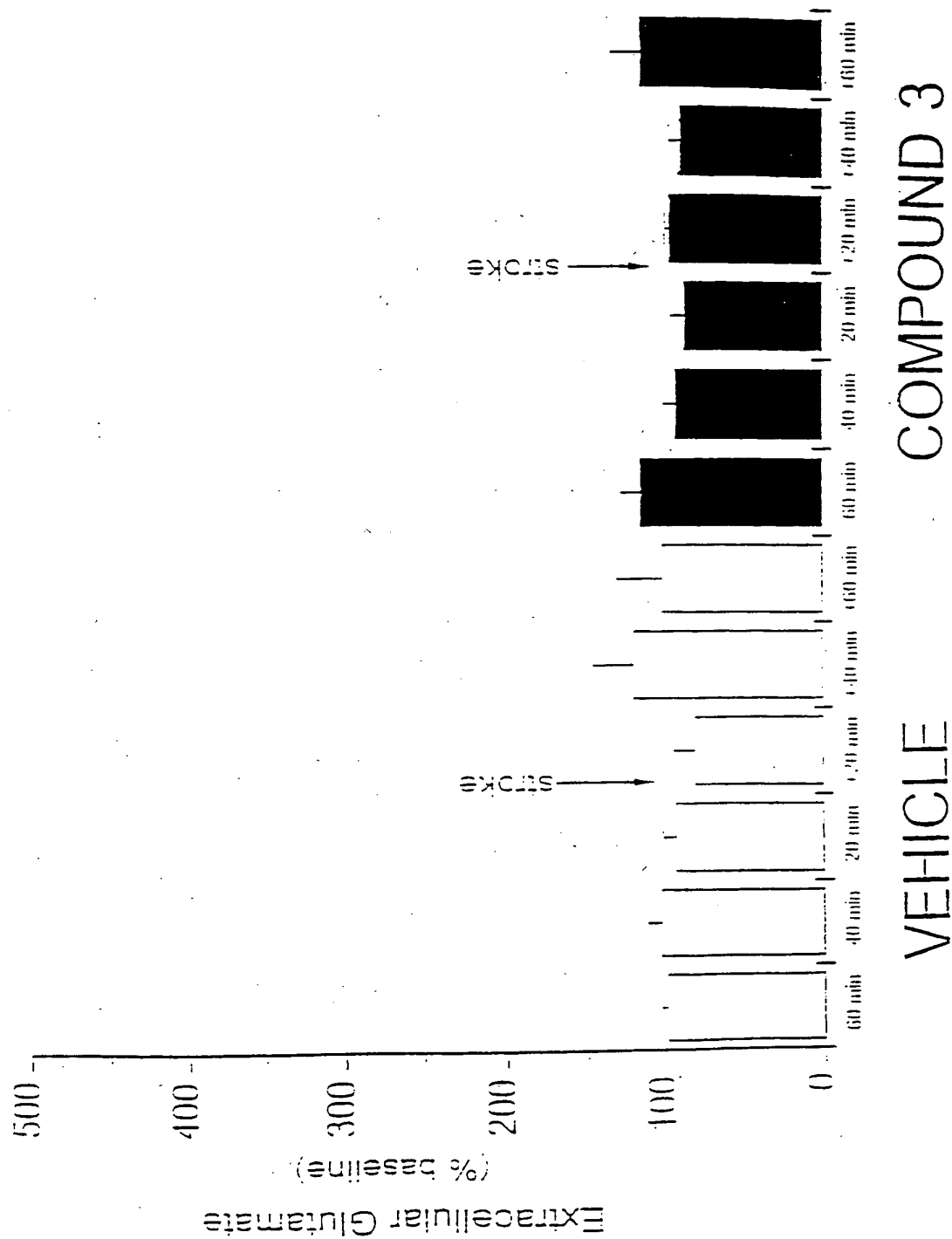
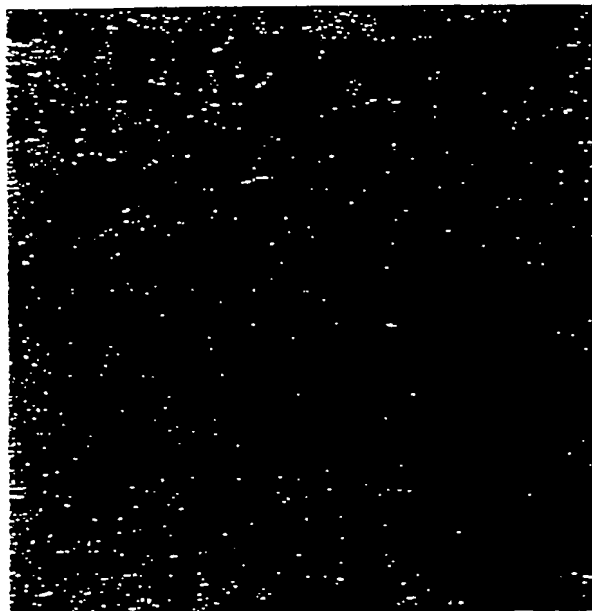


FIGURE 9

Compound 3 Administration Following Sciatic Nerve Crush



Vehicle Polymer

FIGURE 10(a)



Compound 3 Polymer
2 ug drug / day

FIGURE 10(b)

Compound 3 Protects Against MPTP Toxicity in C57BL/6 Mice

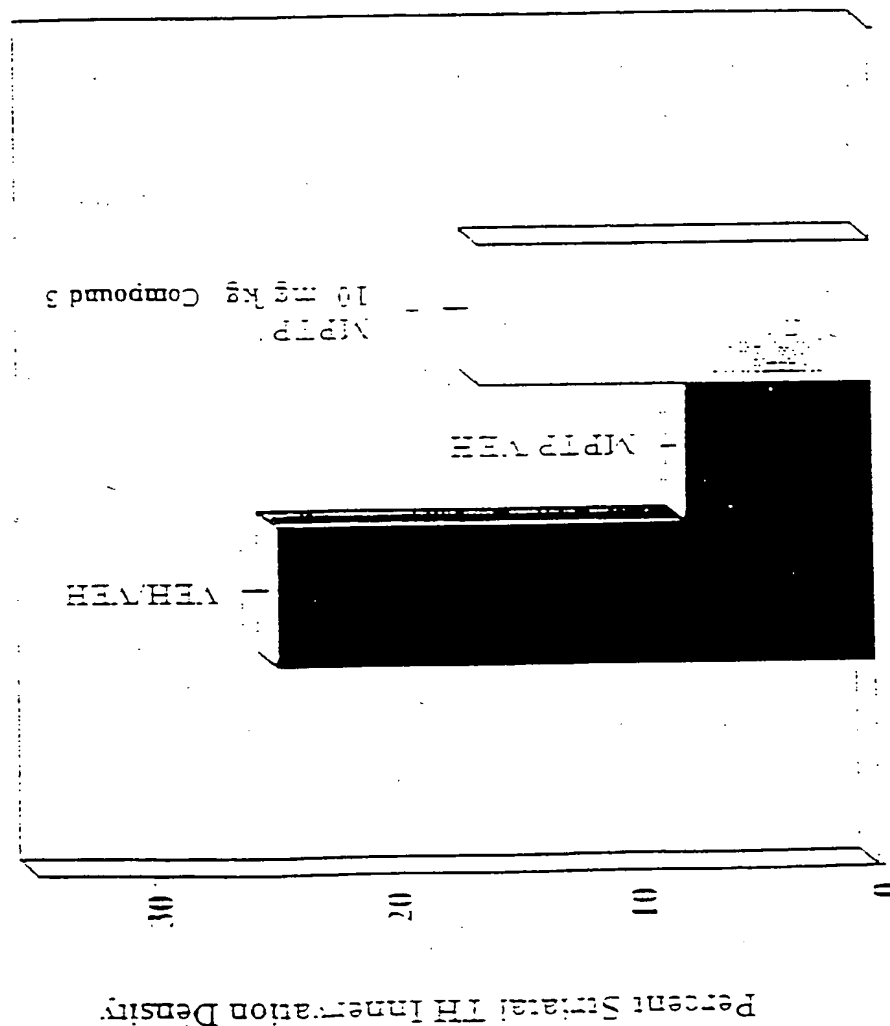


FIGURE 11

Compound 3 is Neuroprotective in a Dynorphin-Induced Spinal Cord Injury Model

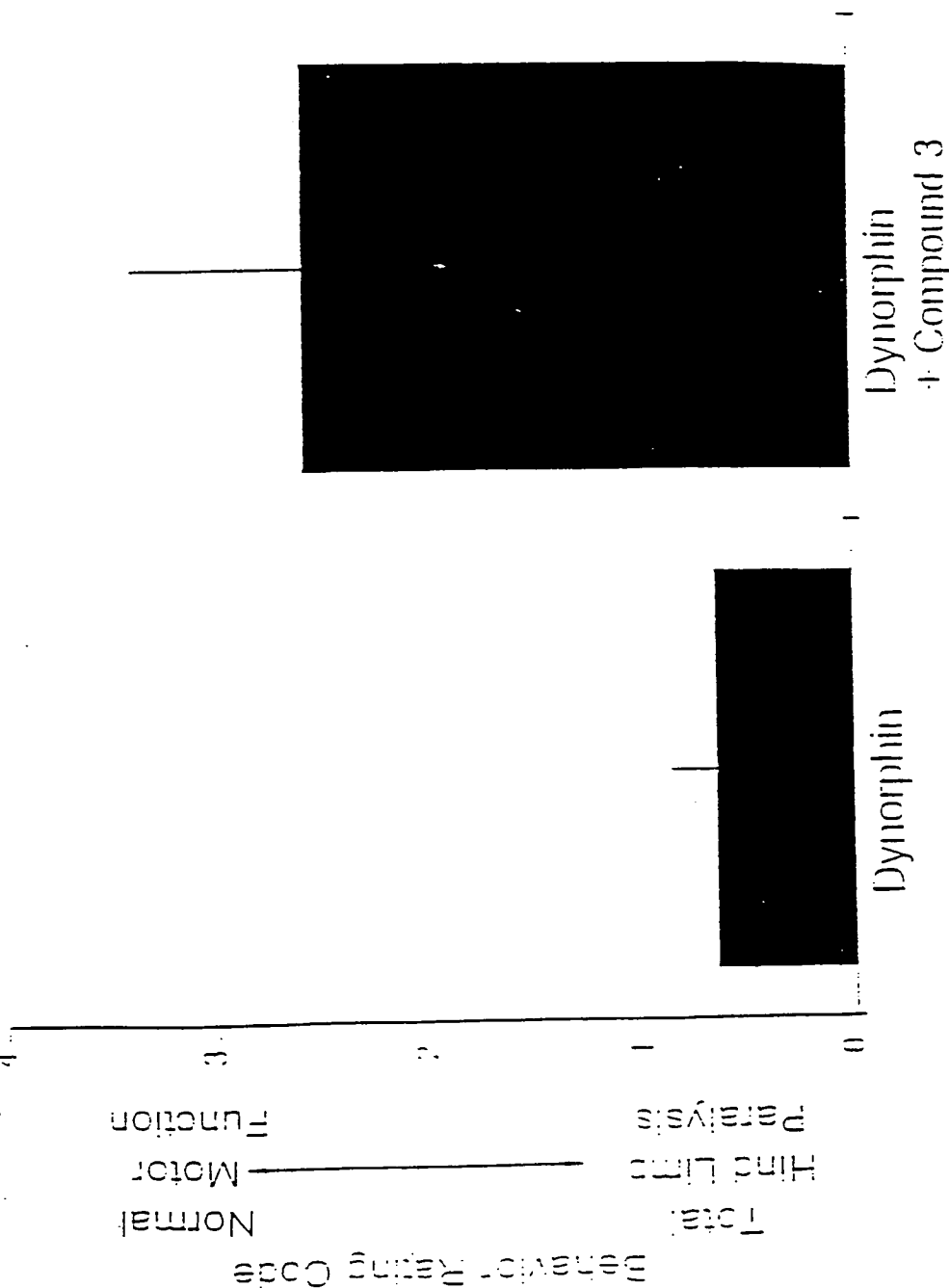


FIGURE 12

Compound 3 is Neuroprotective in a

Spinal Cord Culture Model of ALS

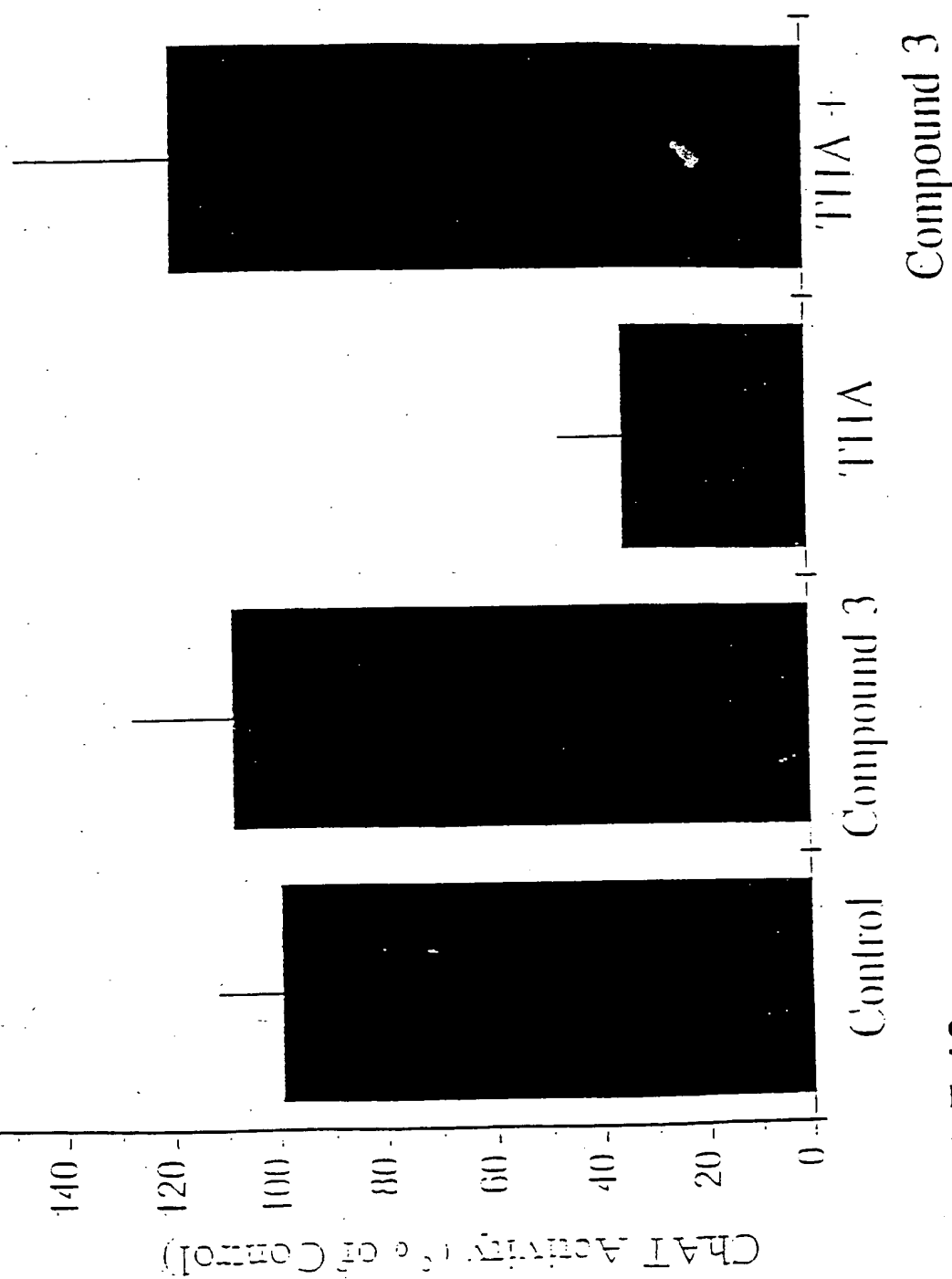


FIGURE 13

Compound 3 is neuroprotective in spinal cord organotypic culture model of ALS

dose response study

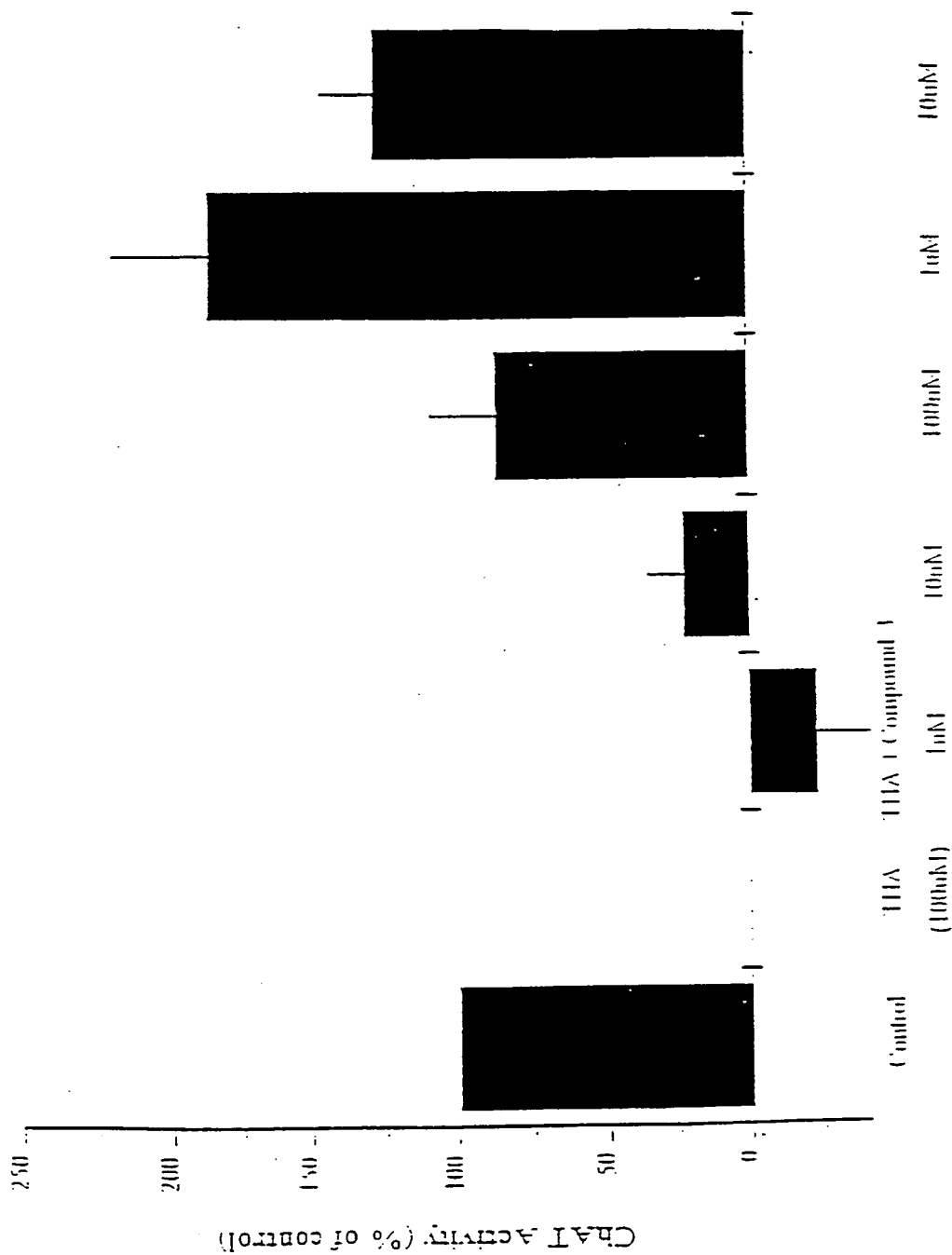


FIGURE 14

Compound 3

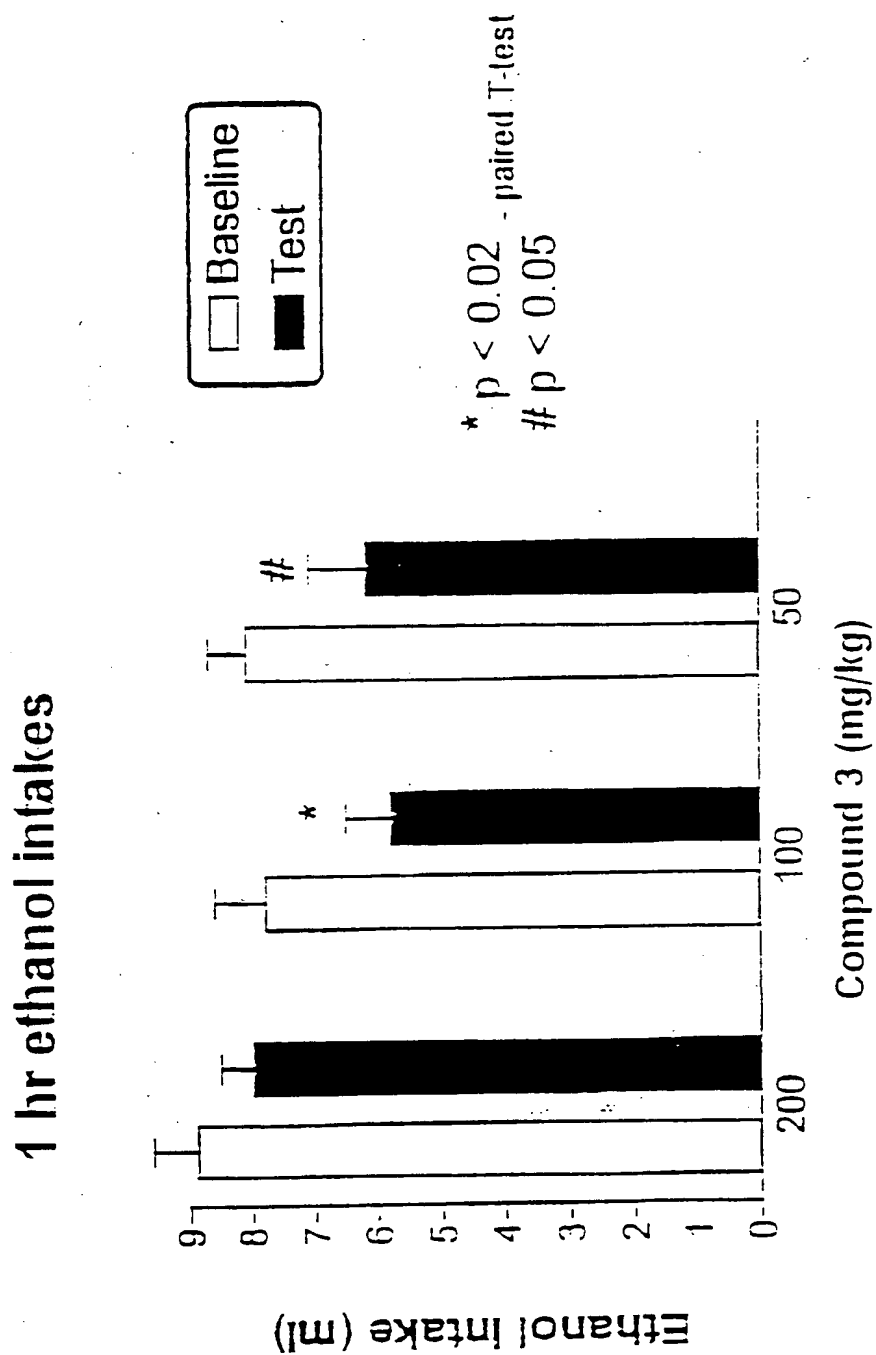


FIGURE 15

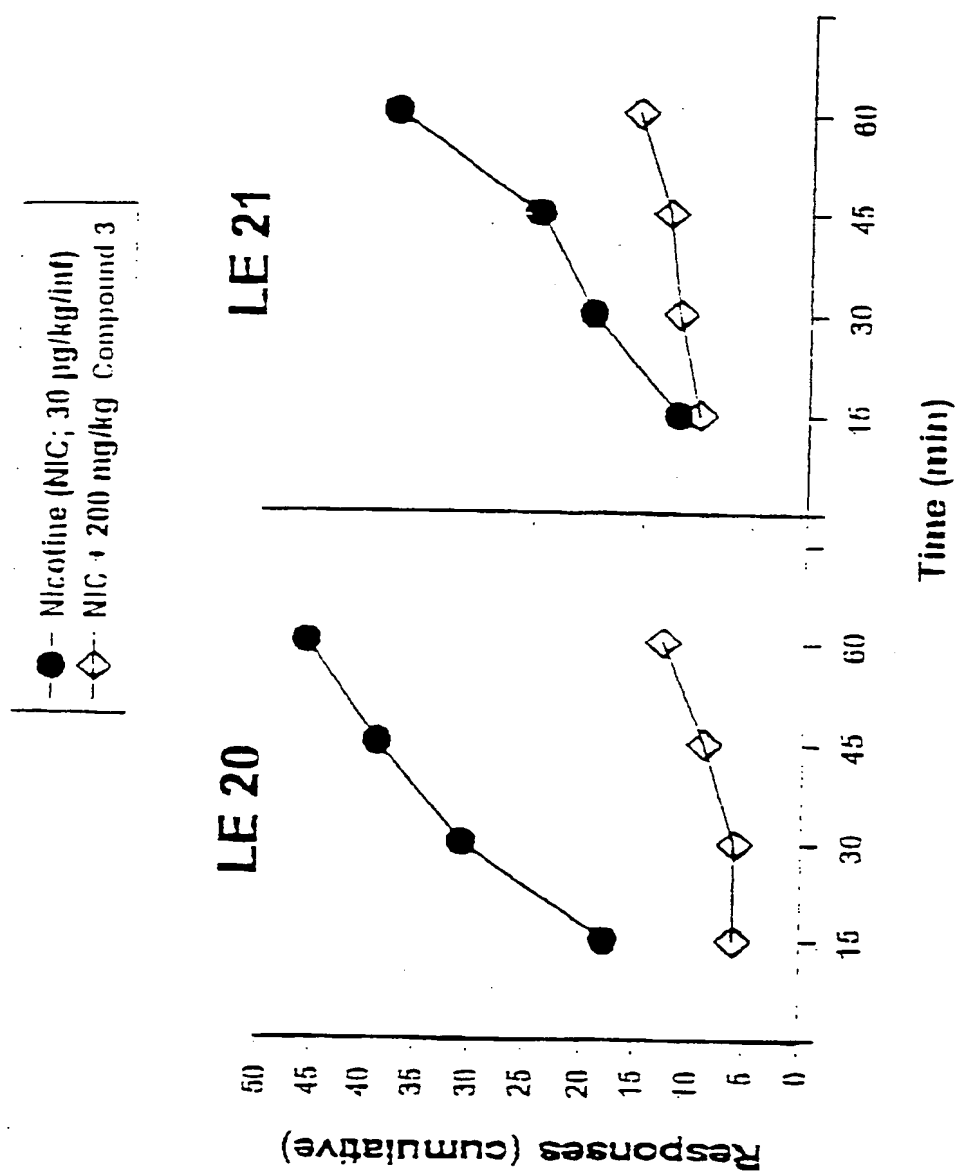


FIGURE 16

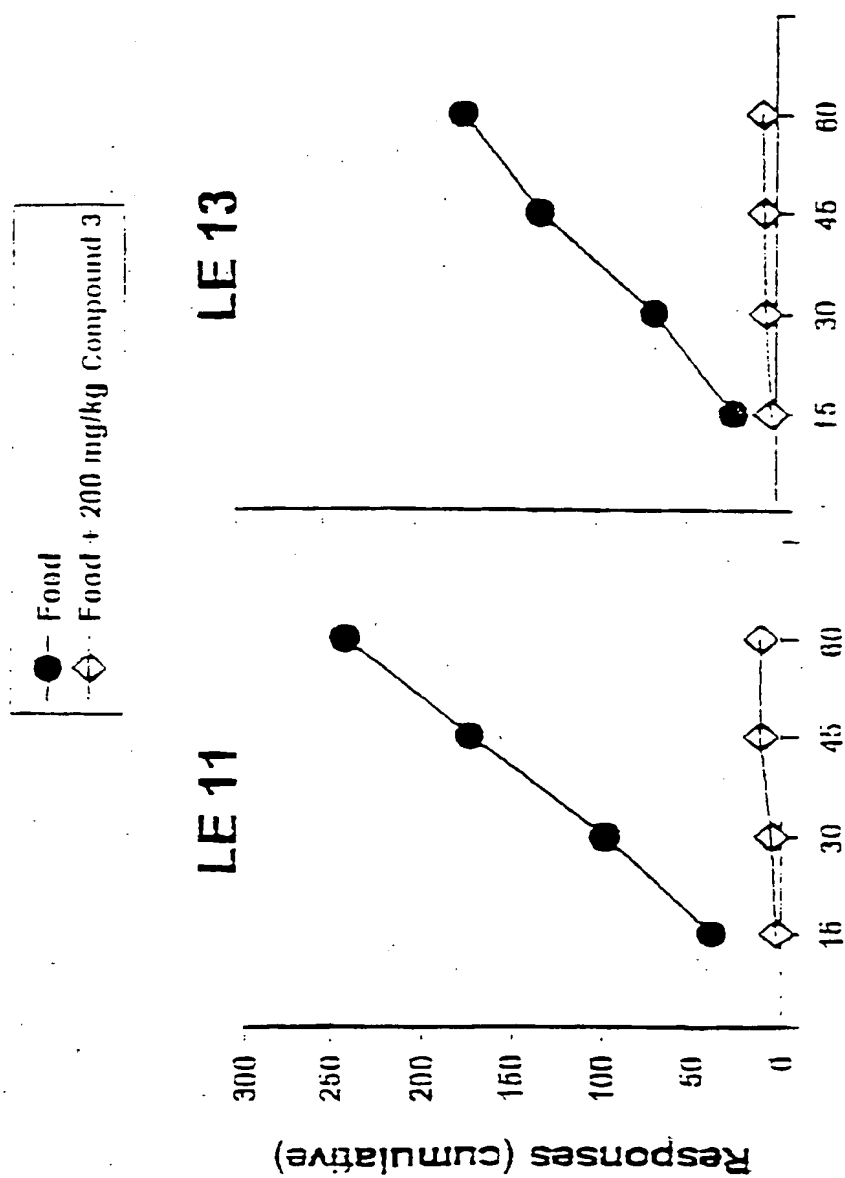
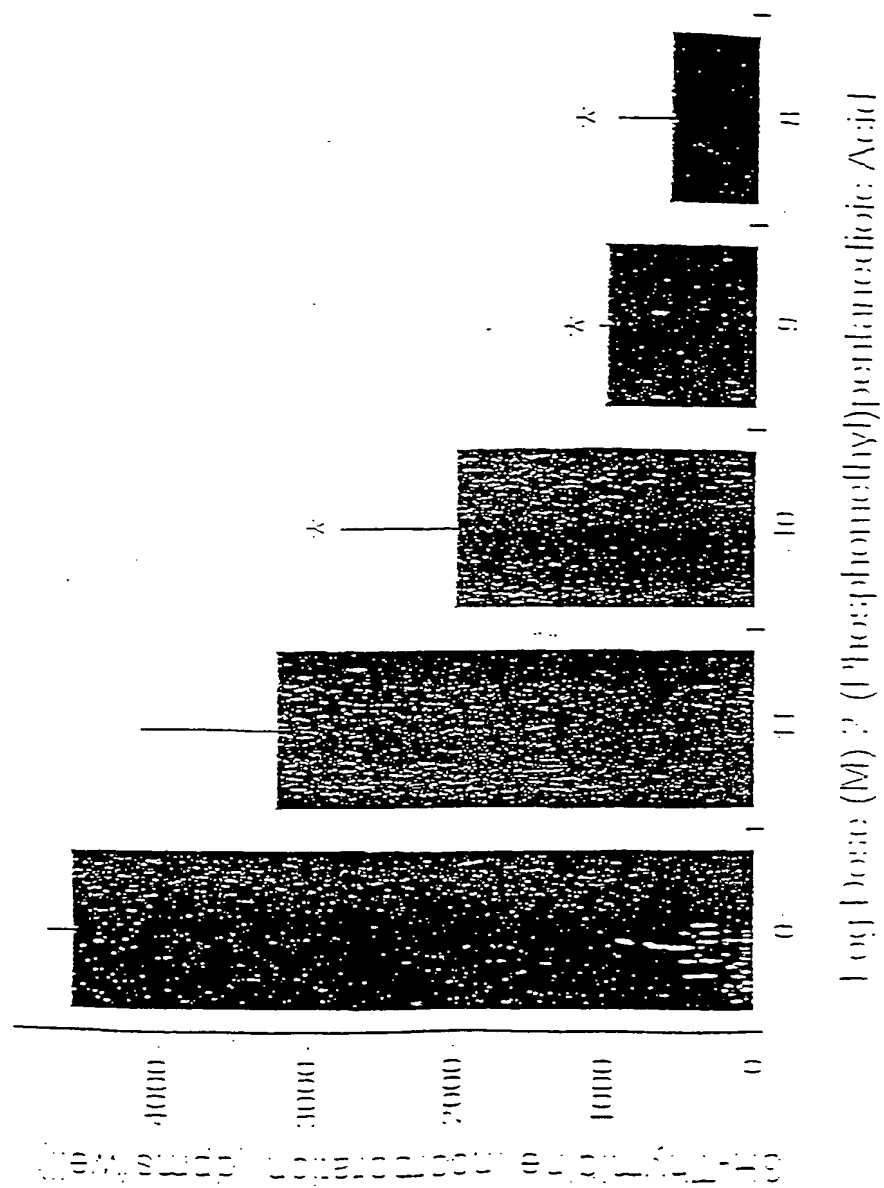


FIGURE 17

**FIGURE 18**

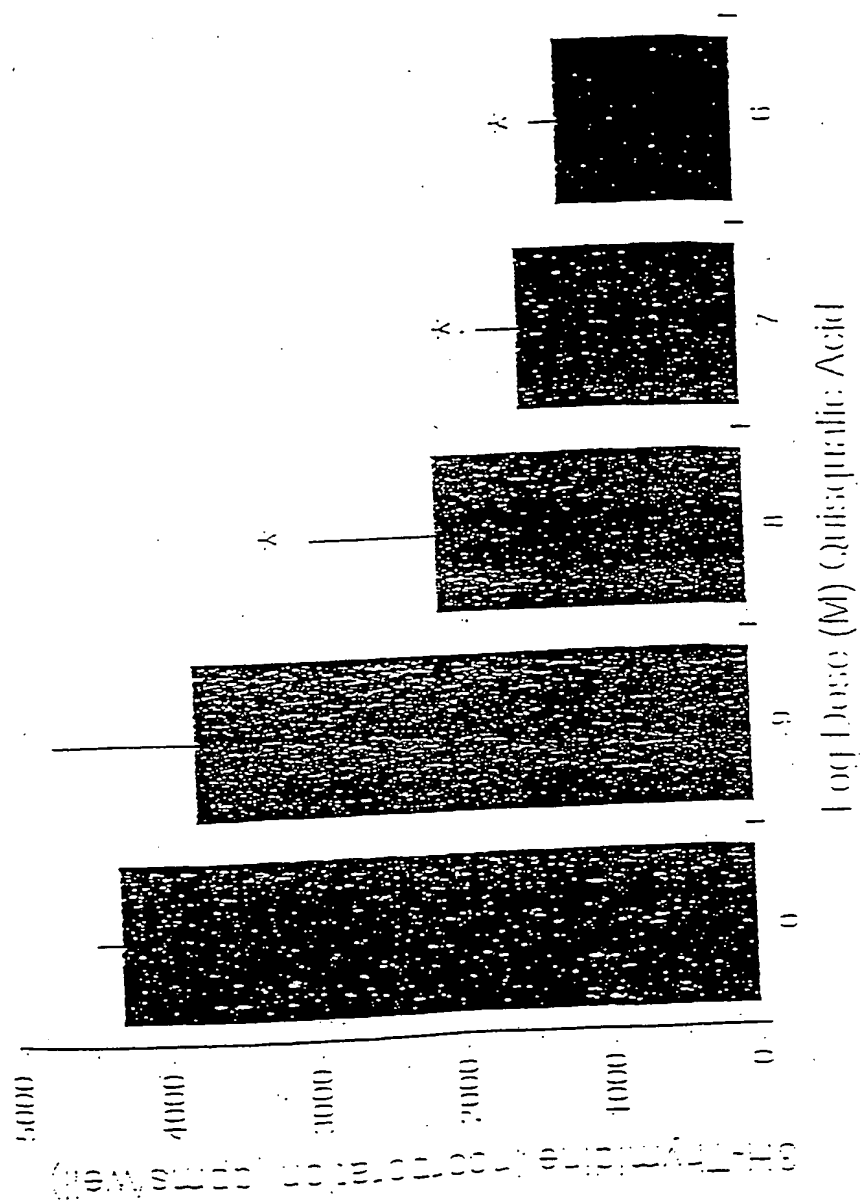


FIGURE 19

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/27175

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07F9/30 A61K31/66 C07F9/58 C07F9/32 C07F9/40
C07F9/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JACKSON P. F.: "Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase N-acetylated alpha-linked acidic dipeptidase" JOURNAL OF MEDICINAL CHEMISTRY., vol. 39, no. 2, - 10 January 1996 pages 619-622, XP002098729 WASHINGTON US see the whole document	1-67
X	WO 97 48400 A (GUILFORD PHARMACEUTICALS INC.) 24 December 1997 see page 72	1-67
P, X	WO 98 13044 A (GUILFORD PHARMACEUTICALS INC.) 2 April 1998 cited in the application see page 21, line 21 and page 84, line 20 --- -/--	1-67

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

1 April 1999

Date of mailing of the international search report

16/04/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Beslier, L

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/27175

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 98 13046 A (GUILFORD PHARMACEUTICALS INC.) 2 April 1998 cited in the application see claims 1, 2, 5-10, 42-49 and 79-85 -----	1-67

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/27175

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9748400 A	24-12-1997	US 5672592 A	30-09-1997
		US 5795877 A	18-08-1998
		US 5863536 A	26-01-1999
		AU 3513597 A	07-01-1998
		AU 3590697 A	07-01-1998
		WO 9748399 A	24-12-1997
		US 5880112 A	09-03-1999
		AU 4151897 A	17-04-1998
		WO 9813044 A	02-04-1998
		AU 3982197 A	30-12-1998
		AU 4067797 A	17-04-1998
		WO 9813046 A	02-04-1998
		WO 9853812 A	03-12-1998
WO 9813044 A	02-04-1998	US 5824662 A	20-10-1998
		US 5795877 A	18-08-1998
		US 5863536 A	26-01-1999
		AU 4067797 A	17-04-1998
		AU 4151897 A	17-04-1998
		WO 9813046 A	02-04-1998
		AU 3513597 A	07-01-1998
		AU 3590697 A	07-01-1998
		WO 9748399 A	24-12-1997
		WO 9748400 A	24-12-1997
		AU 6945198 A	30-10-1998
		WO 9845256 A	15-10-1998
		AU 7100298 A	30-10-1998
		WO 9845257 A	15-10-1998
		AU 6972398 A	13-11-1998
		WO 9847906 A	29-10-1998
		AU 3982197 A	30-12-1998
		WO 9853812 A	03-12-1998
WO 9813046 A	02-04-1998	US 5824662 A	20-10-1998
		AU 4067797 A	17-04-1998
		AU 4151897 A	17-04-1998
		WO 9813044 A	02-04-1998
		AU 6972398 A	13-11-1998
		WO 9847906 A	29-10-1998
		AU 3590697 A	07-01-1998
		AU 3982197 A	30-12-1998
		WO 9748399 A	24-12-1997
		WO 9853812 A	03-12-1998
		AU 3513597 A	07-01-1998
		WO 9748400 A	24-12-1997

DISSOCIATED CULTURE PROTOCOL

For papain
dissociation:

4 mg Cysteine (C-
8277);
25 ml dissoc. EBSS;
250 μ l Papain stock
(Worthington
LS003126);
place in 37°C
waterbath until
clear.

For DNAase treatment:

DNAase, 5 ml

4.5 ml dissoc. EBSS;
500 μ l "10 and 10" stock;
50 μ l DNAase stock.

"10 and 10", 5 ml

4.5 ml of EBSS;
500 μ l "10 and 10" stock.

II. COAT DISHES

Use poly-d-lysine stock at 1:100 dilution to coat
24-well plates (0.5 ml/well) or at 1:10 dilution
to coat 35 mm glass cover slips (1.0
ml/coverslip).

Leave until end of dissection.

THIS PAGE BLANK (USPTO)



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07F 9/30, A61K 31/66, C07F 9/58, 9/32, 9/40, 9/44		A1	(11) International Publication Number: WO 99/33847
			(43) International Publication Date: 8 July 1999 (08.07.99)
(21) International Application Number: PCT/US98/27175 (22) International Filing Date: 21 December 1998 (21.12.98) (30) Priority Data: 09/002,147 31 December 1997 (31.12.97) US (71) Applicant: GUILFORD PHARMACEUTICALS INC. [US/US]; 6611 Tributary Street, Baltimore, MD 21224 (US). (72) Inventors: JACKSON, Paul, F.; 102 Glenmore Court, Bel Air, MD 21014 (US). LI, Weixing, Apartment E, 40 Leatherwood Place, Baltimore, MD 21237 (US). MACLIN, Keith, M.; 5005 Remmel Avenue, Baltimore, MD 21206 (US). TAYS, Kevin, L.; Apartment 1415, 6947 Old Waterloo Road, Elkridge, MD 21227 (US). TSUKAMOTO, Takashi; 4J Cameron Court, Baltimore, MD 21236 (US). SLUSHER, Barbara, S.; 7424 Longfield Drive, Kingsville, MD 21087 (US). (74) Agent: NATH, Gary, M.; Nath & Associates, 6th floor, 1030 15th Street, Washington, DC 20005 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>With amended claims.</i>	
(54) Title: PHOSPHINIC ALKANOIC ACID DERIVATIVES			
(57) Abstract			
<p>The present invention relates to phosphinic alkanoic acid derivatives that inhibit N-Acetylated α-Linked Acidic Dipeptidase (NAALDase) enzyme activity, pharmaceutical compositions comprising the same, and methods of using the same of inhibit NAALDase activity and to treat glutamate abnormalities and prostate diseases.</p>			

*(Referred to in PCT Gazette No. 38/1999, Section II)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

PHOSPHINIC ALKANOIC ACID DERIVATIVES

This application is a continuation-in-part (CIP) of U.S. Patent Application No. 08/665,775 (Attorney Docket No. 23060), filed June 17, 1996, Slusher et al., entitled "Methods of Cancer Treatment Using NAALADase Inhibitors"; U.S. Patent Application No. 08/864,545 (Attorney Docket No. 23060-X), filed May 28, 1997, Slusher et al., entitled "Methods of Cancer Treatment Using NAALADase Inhibitors"; U.S. Patent Application No. 08/718,703 (Attorney Docket No. 23125), filed September 27, 1996, Slusher et al., entitled "Treatment of Global and Focal Ischemia Using NAALADase Inhibitors"; U.S. Patent Application No. 08/884,479 (Attorney Docket No. 23125-X), filed June 27, 1997, Slusher et al., entitled "Pharmaceutical Compositions and Methods of Treating a Glutamate Abnormality and Effecting a Neuronal Activity in an Animal Using NAALADase Inhibitors"; U.S. Patent Application No. 08/899,319 (Attorney Docket No. 23125-XX), filed July 23, 1997, Slusher et al., entitled "Pharmaceutical Compositions and Methods of Treating Compulsive Disorders Using NAALADase Inhibitors"; and U.S. Patent Application No. 08/900,194 (Attorney Docket No. 23060-XX), filed July 29, 1997, Jackson et al., entitled "Inhibitors of NAALADase Enzyme Activity".

BACKGROUND OF THE INVENTION

1. Field of the Invention

5 The present invention relate to phosphinic alkanolic acid derivatives that inhibit N-Acetylated α -Linked Acidic Dipeptidase (NAALADase) enzyme activity, pharmaceutical compositions comprising the same, and methods of using the same to inhibit NAALADase activity and to treat glutamate abnormalities and prostate diseases.

2. Description of the Prior Art

Glutamate Abnormalities

15 Glutamate serves as the predominant excitatory neurotransmitter in the central nervous system (CNS). Neurons release glutamate in great quantities when they are deprived of oxygen, as may occur during an ischemic brain insult such as a stroke or a heart attack. This excess release of glutamate in turn causes over-stimulation (excitotoxicity) of N-methyl-D-aspartate (NMDA), AMPA, Kainate and MGR receptors. When glutamate binds to these receptors, ion channels in the receptors open, permitting flows of ions across their cell membranes, e.g., Ca^{2+} and Na^{+} into the cells and K^{+} out of the cells. These flows of ions, especially the influx of

Ca²⁺, cause over-stimulation of the neurons. The over-stimulated neurons secrete more glutamate, creating a domino-effect which ultimately results in cell death via the production of proteases, lipases and free radicals.

5 Excessive activation of glutamate receptors has been implicated in various neurological diseases and conditions, including epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease, schizophrenia,
10 chronic pain, ischemia and neuronal loss following hypoxia, hypoglycemia, ischemia, trauma, and nervous insult. Recent studies have also advanced a glutamatergic basis for compulsive disorders, particularly drug dependence.

15 As an example, neurophysiological and pathological effects of ethanol have been found to be mediated through the glutamatergic system. Specifically, acute exposure to ethanol disrupts glutamatergic neurotransmission by inhibiting ion flow through channels in glutamate
20 receptors, whereas chronic exposure up-regulates the number of glutamate receptors and thereby increases ion flow. Acute withdrawal from ethanol results in hyperexcitability and seizures in the presence of up-regulated channels, thereby making postsynaptic neurons
25 vulnerable to excitotoxic damage.

Post mortem examinations of histologically normal

brains from alcoholics have shown that chronic alcoholism moderately increases the density of the NMDA subtype of glutamate receptors in the frontal cortex. This up-regulation may represent a stage of ethanol-induced chronic neurotoxicity. As such, neurobiological effects of alcoholism, including intoxication, withdrawal seizures, delirium tremens, Wernicke-Korsakoff syndrome and fetal alcohol syndrome, can be understood as a spectrum of the consequences of ethanol's effect on the glutamatergic system. In this regard, alcoholism may be considered another member of the expanding family of glutamate-related neurological disorders.

The glutamatergic system has also been implicated in the behavioral effects of other abused drugs. For example, studies have shown that glutamatergic antagonists block motor-stimulating activities induced by amphetamine and cocaine, and glutamatergic agonists cause the same stereotypy as that produced by amphetamine. These results represent pharmacological evidence that the expression of the stereotypic effect of psychomotor stimulants involves the glutamatergic system.

Epidemiologic studies have revealed a strong correlation between drug dependence and other compulsive disorders. Additionally, a common genetic anomaly has been found among people with alcoholism, cocaine dependence, nicotine dependence, pathological gambling,

attention deficit disorder (ADD), Tourette's syndrome, compulsive overeating and obesity. Such disorders are believed to be manifestations of the effects of excitotoxicity.

5 Attempts to prevent excitotoxicity by blocking NMDA, AMPA, Kainate and MGR receptors have proven difficult because each receptor has multiple sites to which glutamate may bind. Many of the compositions that are effective in blocking the receptors are also toxic to
10 animals. As such, there is currently no known effective treatment for glutamate abnormalities.

Prostate Cancer

Prostate cancer is the leading form of cancer and the second leading cause of death from cancer for men in
15 the United States. The American Cancer Society has estimated that in 1996 alone, 317,100 new cases of prostate cancer were diagnosed and 41,400 deaths were caused by prostate cancer. The incidence rate of prostate cancer increased 65% between 1980 and 1990, and
20 will continue to rise with improved screening tests and longer life expectancies. While most men used to die of other illnesses before prostate cancer had a chance to develop, higher prostate cancer mortality rates are expected as men live longer and the disease has more time
25 to progress.

In 1993, the molecular cloning of Prostate Specific

Membrane Antigen (PSMA) was reported as a potential prostate carcinoma marker and hypothesized to serve as a target for imaging and cytotoxic treatment modalities for prostate cancer. PSMA antibodies, particularly indium-111 labelled and itrium labelled PSMA antibodies, have been described and examined clinically for the diagnosis and treatment of prostate cancer. PSMA is expressed in prostatic ductal epithelium and is present in seminal plasma, prostatic fluid and urine. In 1996, it was found that the expression of PSMA cDNA confers the activity of NAALADase.

NAALADase Inhibitors

NAAG and NAALADase have been implicated in several human and animal pathological conditions. For example, it has been demonstrated that intra-hippocampal injections of NAAG elicit prolonged seizure activity. More recently, it was reported that rats genetically prone to epileptic seizures have a persistent increase in their basal level of NAALADase activity. These observations support the hypothesis that increased availability of synaptic glutamate elevates seizure susceptibility, and suggest that NAALADase inhibitors may provide anti-epileptic activity.

NAAG and NAALADase have also been implicated in the pathogenesis of ALS and in the pathologically similar animal disease called Hereditary Canine Spinal Muscular

Atrophy (HCSMA). It has been shown that concentrations of NAAG and its metabolites -- NAA, glutamate and aspartate -- are elevated two- to three-fold in the cerebrospinal fluid of ALS patients and HCSMA dogs. Additionally, NAALADase activity is significantly increased (two- to three-fold) in post-mortem spinal cord tissue from ALS patients and HCSMA dogs. As such, NAALADase inhibitors may be clinically useful in curbing the progression of ALS if increased metabolism of NAAG is responsible for the alterations of CSF levels of these acidic amino acids and peptides.

Abnormalities in NAAG levels and NAALADase activity have also been documented in post-mortem schizophrenic brain, specifically in the prefrontal and limbic brain regions.

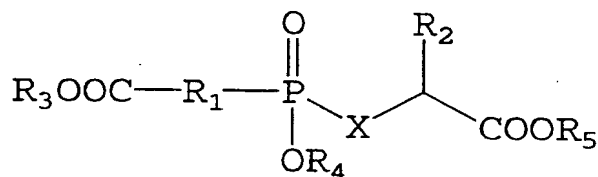
The findings described above suggest that NAALADase inhibitors could be useful in treating glutamate abnormalities. In fact, the results of studies conducted by the inventors confirm that NAALADase inhibitors are effective in treating glutamate abnormalities (particularly stroke, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), spinal cord injury, alcoholism and nicotine dependence), as well as prostate diseases (particularly prostate cancer).

While a few NAALADase inhibitors have been identified, they have only been used in non-clinical

research. Examples of such inhibitors include general metallopeptidase inhibitors such as o-phenanthroline, metal chelators such as EGTA and EDTA, and peptide analogs such as quisqualic acid and β -NAAG. Accordingly,
 5 a need exists for new NAALADase inhibitors, as well as pharmaceutical compositions and methods using such new and known NAALADase inhibitors to treat glutamate abnormalities and prostate diseases.

10 SUMMARY OF THE INVENTION

The present invention relates to a compound of formula I:



I

or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

20 X is CR_6R_7 , O or NR_8 ;

R_1 is selected from the group consisting of C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl and Ar, wherein said R_1 is unsubstituted or
 25 substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl,

C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain

alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

The present invention also relates to a method of treating a glutamate abnormality in an animal, comprising administering to said animal an effective amount of a compound of formula I.

The present invention further relates to a method of effecting a neuronal activity in an animal, comprising administering to said animal an effective amount of a compound of formula I.

Additionally, the present invention relates to a method of treating a compulsive disorder, comprising administering to a patient in need thereof an effective amount of a compound of formula I.

Furthermore, the present invention relates to a method of treating a prostate disease in an animal, comprising administering to said animal an effective amount of a compound of formula I.

Moreover, the present invention relates to a method of inhibiting NAALADase activity in an animal, comprising administering to said animal an effective amount of a compound of formula I.

Finally, the present invention relates to a pharmaceutical composition comprising:

(i) an effective amount of a compound of formula I;

and

(ii) a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

5 FIG. 1 is a bar graph plotting *in vitro* toxicity of ischemic insult (potassium cyanide and 2-deoxyglucose) against various doses of 2-(phosphonomethyl)pentanedioic acid with which cortical cell cultures were treated.

10 FIG. 2 is a bar graph plotting *in vitro* toxicity against various doses of NAAG to which cortical cell cultures were exposed.

FIG. 3 is a bar graph plotting *in vitro* toxicity following treatment with 2-(phosphonomethyl)pentanedioic acid, against various doses of NAAG to which cortical cell cultures were exposed.

15 FIG. 4 is a bar graph plotting *in vitro* toxicity of ischemic insult against various times at which cortical cell cultures were treated with 2-(phosphonomethyl)-pentanedioic acid.

20 FIG. 5 is a bar graph plotting *in vivo* cortical injury volume against various doses of 2-(phosphonomethyl)pentanedioic acid with which rats were treated after sustaining middle cerebral artery occlusion.

25 FIG. 6 is a bar graph plotting *in vivo* total brain infarct volume of rats against various times at which the rats are treated with 2-(phosphonomethyl)pentanedioic

acid after sustaining middle cerebral artery occlusion.

FIG. 7 is a bar graph plotting *in vivo* extracellular glutamate increases in the striatum of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

FIG. 8 is a bar graph plotting *in vivo* extracellular glutamate increases in the parietal cortex of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

FIG. 9 is a bar graph plotting *in vivo* extracellular glutamate increases in the frontal cortex of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

FIG. 10(a) is a photomicrograph of mouse sciatic nerve treated with a vehicle following cryolesion.

FIG. 10(b) is a photomicrograph of mouse sciatic nerve treated with 2-(phosphonomethyl)pentanedioic acid following cryolesion.

FIG. 11 is a bar graph plotting percent striatal TH innervation density against the treatment of mice with vehicle alone, vehicle following MPTP, or 2-(phosphonomethyl)pentanedioic acid following MPTP.

FIG. 12 is a bar graph plotting the neurological function code against the treatment of rats with dynorphin A alone or 2-(phosphonomethyl)pentanedioic acid with dynorphin A.

FIG. 13 is a bar graph plotting the ChAT activity of rat spinal cord organotypic cultures against the treatment of the cultures with 2-(phosphonomethyl)-pentanedioic acid alone, threohydroxyaspartate (THA) alone, or THA with 2-(phosphonomethyl)pentanedioic acid.

FIG. 14 is a bar graph plotting the ChAT activity of rat spinal cord organotypic cultures against various doses of 2-(phosphonomethyl)pentanedioic acid with which the cultures were treated in the presence of THA.

FIG. 15 is a bar graph plotting the ethanol intake of alcohol-preferring rats against various doses of 2-(phosphonomethyl)pentanedioic acid with which the rats were treated.

FIG. 16 is a graph plotting the cumulative nicotine intake of rats during a 1 hour test session, before which the rats had been trained to self-administer nicotine and pretreated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid.

FIG. 17 is a graph plotting the cumulative food intake of rats during a 1 hour test session, before which the rats had been trained to self-administer nicotine and pretreated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid.

FIG. 18 is a bar graph plotting *in vitro* cancer cell growth against various doses of quisqualic acid with which LNCAP cells were treated.

FIG. 19 is a bar graph plotting *in vitro* cancer cell growth against various doses of 2-(phosphonomethyl)-pentanedioic acid with which LNCAP cells were treated.

5

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Attention Deficit Disorder" refers to a disorder characterized by developmentally inappropriate inattention and impulsivity, with or without hyperactivity. Inattention means a failure to finish tasks started, easy distractibility, seeming lack of attention, and difficulty concentrating on tasks requiring sustained attention. Impulsivity means acting before thinking, difficulty taking turns, problems organizing work, and constant shifting from one activity to another. Hyperactivity means difficulty staying seated and sitting still, and running or climbing excessively.

"Compound 3" refers to 2-(phosphonomethyl)pentanedioic acid (PMPA).

"Compulsive disorder" refers to any disorder characterized by irresistible impulsive behavior. Examples of compulsive disorders include without limitation drug dependence, eating disorders, pathological gambling, ADD and Tourette's syndrome.

"Drug dependence" refers to a psychologic addiction

or a physical tolerance to a drug. Tolerance means a need to increase the dose progressively in order to produce the effect originally achieved by smaller amounts.

5 "Eating disorder" refers to compulsive overeating, obesity or severe obesity. Obesity means body weight of 20% over standard height-weight tables. Severe obesity means over 100% overweight.

10 "Glutamate abnormality" refers to any disease, disorder or condition in which glutamate is implicated, including pathological conditions involving elevated levels of glutamate. Examples of glutamate abnormalities include epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS),
15 Huntington's Disease, schizophrenia, chronic pain, ischemia, neuronal insult and compulsive disorders.

"Glutamate modulator" refers to any composition of matter which alone or in combination with another agent affects the level of glutamate in an animal.

20 "Inhibition", in the context of enzymes, refers to reversible enzyme inhibition such as competitive, uncompetitive and non-competitive inhibition. Competitive, uncompetitive and non-competitive inhibition can be distinguished by the effects of an inhibitor on
25 the reaction kinetics of an enzyme. Competitive inhibition occurs when the inhibitor combines reversibly

with the enzyme in such a way that it competes with a normal substrate for binding at the active site. The affinity between the inhibitor and the enzyme may be measured by the inhibitor constant, K_i , which is defined as:

$$K_i = \frac{[E][I]}{[EI]}$$

10

wherein [E] is the concentration of the enzyme, [I] is the concentration of the inhibitor, and [EI] is the concentration of the enzyme-inhibitor complex formed by the reaction of the enzyme with the inhibitor. Unless otherwise specified, K_i as used herein refers to the affinity between the inventive compounds and NAALADase. "IC₅₀" is a related term used to define the concentration or amount of a compound which is required to cause a 50% inhibition of the target enzyme.

20

"Ischemia" refers to localized tissue anemia due to obstruction of the inflow of arterial blood. Global ischemia occurs when blood flow to the entire brain ceases for a period of time, such as may result from cardiac arrest. Focal ischemia occurs when a portion of the brain is deprived of its normal blood supply, such as may result from thromboembolytic occlusion of a cerebral vessel, traumatic head injury, edema or brain tumor. Even if transient, both global and focal ischemia can

25

produce widespread neuronal damage. Although nerve tissue damage occurs over hours or even days following the onset of ischemia, some permanent nerve tissue damage may develop in the initial minutes following cessation of blood flow to the brain. Much of this damage is attributed to glutamate toxicity and secondary consequences of reperfusion of the tissue, such as the release of vasoactive products by damaged endothelium, and the release of cytotoxic products, such as free radicals and leukotrienes, by the damaged tissue.

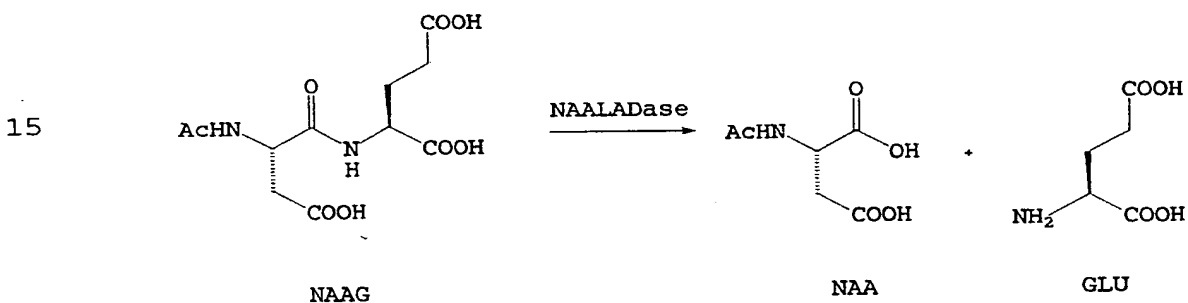
"Isomers" refer to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the arrangement or configuration of the atoms. "Stereoisomers" are isomers that differ only in the arrangement of the atoms in space. "Enantiomers" are a pair of stereoisomers that are non-superimposable mirror images of each other. "Diastereoisomers" are stereoisomers which are not mirror images of each other. "Racemic mixture" means a mixture containing equal parts of individual enantiomers. "Non-racemic mixture" is a mixture containing unequal parts of individual enantiomers or stereoisomers.

"NAAG" refers to N-acetyl-aspartyl-glutamate, an important peptide component of the brain, with levels comparable to the major inhibitor neurotransmitter gamma-aminobutyric acid (GABA). NAAG is neuron-specific,

present in synaptic vesicles and released upon neuronal stimulation in several systems presumed to be glutamatergic. Studies suggest that NAAG may function as a neurotransmitter and/or neuromodulator in the central nervous system, or as a precursor of the neurotransmitter glutamate.

"NAALADase" refers to N-acetylated α -linked acidic dipeptidase, a membrane-bound metallopeptidase which catabolizes NAAG to N-acetylaspartate (NAA) and glutamate:

Catabolism of NAAG by NAALADase



20

NAALADase shows a high affinity for NAAG with a K_m of 540 nM. If NAAG is a bioactive peptide, then NAALADase may serve to inactivate NAAG'S synaptic action. Alternatively, if NAAG functions as a precursor for glutamate, the primary function of NAALADase may be to regulate synaptic glutamate availability.

25

"Nervous function" refers to the various functions of the nervous system, which among other things provide an awareness of the internal and external environments of the body, make possible voluntary and reflex activities between the various structural elements of the organism, and balance the organism's response to environmental changes.

"Nervous insult" refers to any damage to nervous tissue and any disability or death resulting therefrom. The cause of nervous insult may be metabolic, toxic, neurotoxic, iatrogenic, thermal or chemical, and includes without limitation ischemia, hypoxia, cerebrovascular accident, trauma, surgery, pressure, mass effect, hemorrhage, radiation, vasospasm, neurodegenerative disease, neurodegenerative process, infection, Parkinson's disease, ALS, myelination/demyelination process, epilepsy, cognitive disorder, glutamate abnormality and secondary effects thereof. Currently, there is no known effective treatment for nervous tissue damage.

"Nervous tissue" refers to the various components that make up the nervous system, including without limitation neurons, neural support cells, glia, Schwann cells, vasculature contained within and supplying these structures, the central nervous system, the brain, the brain stem, the spinal cord, the junction of the central

nervous system with the peripheral nervous system, the peripheral nervous system and allied structures.

"Neuroprotective" refers to the effect of reducing, arresting or ameliorating nervous insult, and protecting, resuscitating or reviving nervous tissue which has suffered nervous insult.

"Pathological gambling" is a condition characterized by a preoccupation with gambling. Similar to psychoactive substance abuse, its effects include development of tolerance with a need to gamble progressively larger amounts of money, withdrawal symptoms, and continued gambling despite severe negative effects on family and occupation.

"Pharmaceutically acceptable salt" refers to a salt of the inventive compounds which possesses the desired pharmacological activity and which is neither biologically nor otherwise undesirable. The salt can be formed with inorganic acids such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate,

oxalate, thiocyanate, tosylate and undecanoate. Examples of a base salt include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with
5 organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine. The basic nitrogen-containing groups can be quarternized with agents including lower alkyl halides such as methyl, ethyl, propyl and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl,
10 diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

15 "Pharmaceutically acceptable prodrug" refers to a derivative of the inventive compounds which undergoes biotransformation prior to exhibiting its pharmacological effect(s). The prodrug is formulated with the objective(s) of improved chemical stability, improved
20 patient acceptance and compliance, improved bioavailability, prolonged duration of action, improved organ selectivity, improved formulation (e.g., increased hydrosolubility), and/or decreased side effects (e.g., toxicity). The prodrug can be readily prepared from the
25 inventive compounds using methods known in the art, such as those described by *Burger's Medicinal Chemistry and*

Drug Chemistry, Fifth Ed., Vol. 1, pp. 172-178, 949-982 (1995). For example, the inventive compounds can be transformed into prodrugs by converting one or more of the hydroxy or carboxy groups into esters.

5 "Tourette's syndrome" refers to an autosomal multiple tic disorder characterized by compulsive swearing, multiple muscle tics and loud noises. Tics are brief, rapid, involuntary movements that can be simple or complex; they are stereotyped and repetitive, but not
10 rhythmic. Simple tics, such as eye blinking, often begin as nervous mannerisms. Complex tics often resemble fragments of normal behavior.

"Treating" refers to:

(i) preventing a disease, disorder or condition
15 from occurring in an animal which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and

20 (iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

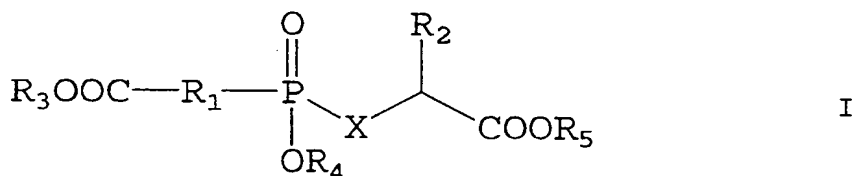
In relation to drug dependence, "treating" refers to suppressing the psychologic addiction or physical
25 tolerance to the drug of abuse, and relieving or preventing a withdrawal syndrome resulting from the drug

dependence.

"Withdrawal syndrome" refers to a disorder characterized by untoward physical changes that occur when the drug is discontinued or when its effect is counteracted by a specific antagonist.

COMPOUNDS OF THE PRESENT INVENTION

The present invention relates to a compound of formula I:



or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉

alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉,
5 straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected
10 from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

15 Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranlyl, tetrahydropyranylyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or
20 substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy,
25 benzyloxy, and amino.

In a preferred embodiment, X is CH₂.

In a more preferred embodiment, R_2 is $-(CH_2)_2COOR_9$; and R_9 is selected from the group consisting of hydrogen, C_1-C_9 straight or branched chain alkyl, C_2-C_9 straight or branched chain alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl and Ar, wherein said R_9 is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 straight or branched chain alkyl, C_2-C_6 straight or branched chain alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

In the most preferred embodiment, R_3 , R_4 , R_5 , and R_9 are hydrogen.

Preferred compounds of formula I are selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxybutyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]-
pentanedioic acid;
2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;
5 2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;
2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;
2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-
10 phosphinyl)methyl]pentanedioic acid; and
pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

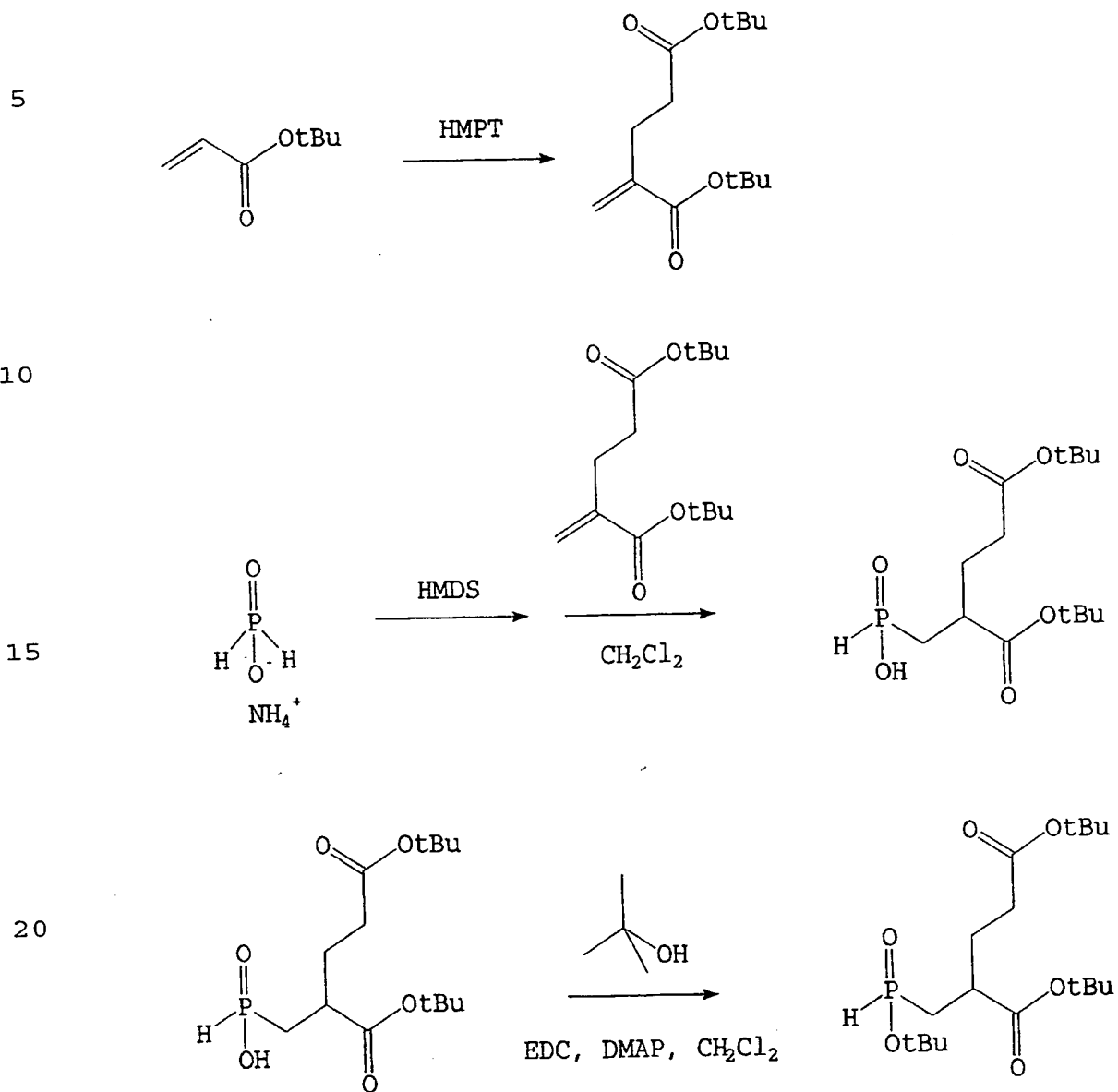
The most preferred compound of formula I is 2-[[[(2-
carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid,
15 or a pharmaceutically acceptable salt, hydrate or prodrug
thereof.

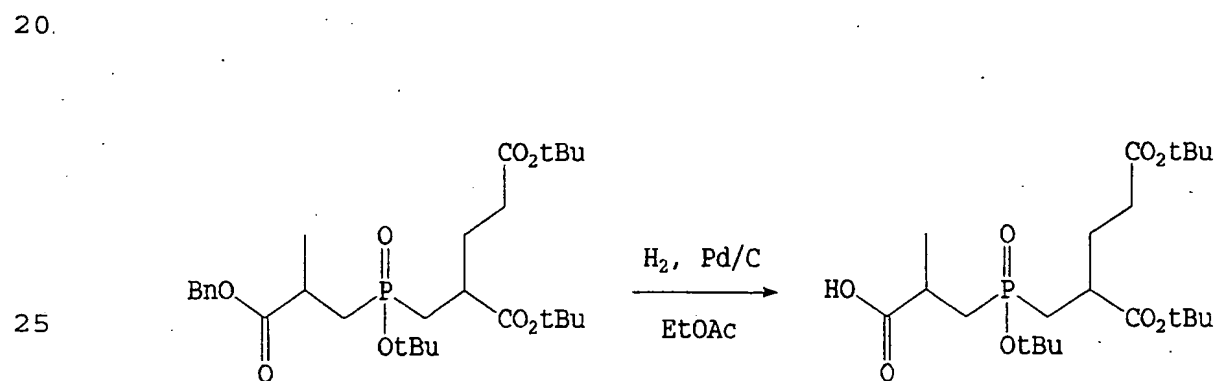
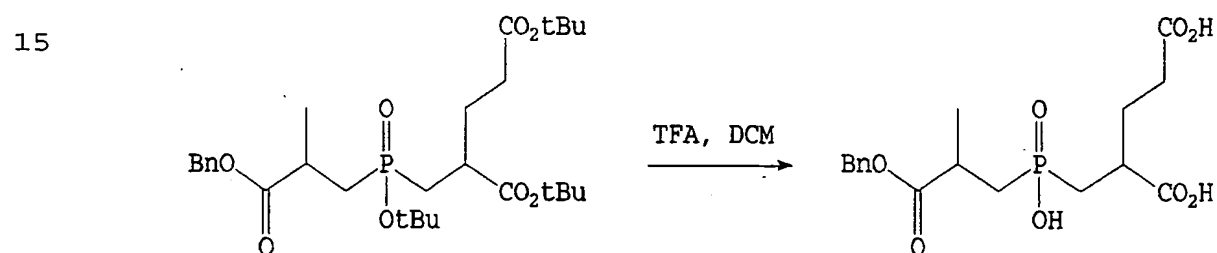
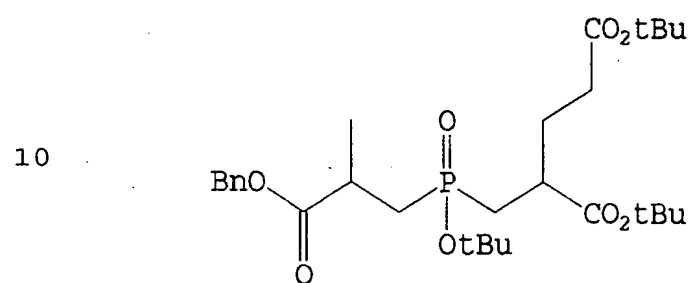
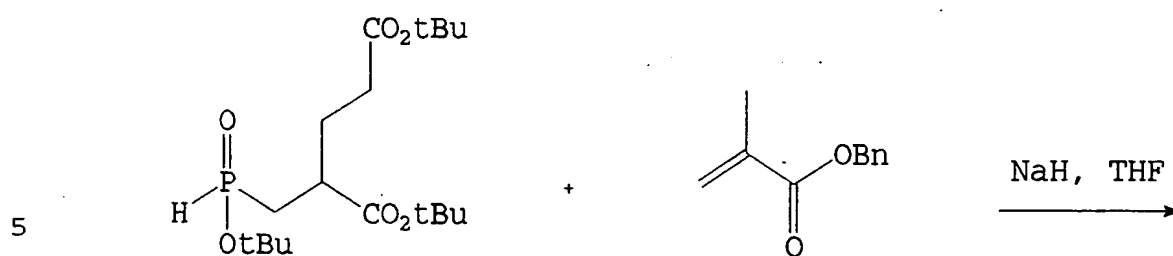
The compounds of the present invention possess one
or more asymmetric center(s) and thus can be produced as
mixtures (racemic and non-racemic) of stereoisomers, or
20 as individual R- and S-stereoisomers. The individual
stereoisomers may be obtained by using an optically
active starting material, by resolving a racemic or non-
racemic mixture of an intermediate at some appropriate
stage of synthesis, or by resolving a compound of formula
25 I.

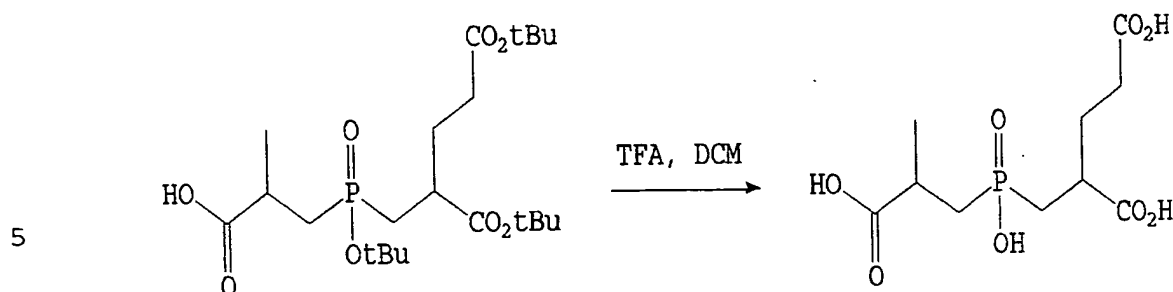
Synthesis of Compounds

The compounds of the present invention can be readily prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways depicted below in Scheme I. Precursor compounds can be prepared by methods known in the art, such as those described by Jackson et al., *J. Med. Chem.*, Vol. 39, No. 2, pp. 619-622 (1996) and Froestl et al., *J. Med. Chem.*, Vol. 38, pp. 3313-3331 (1995).

10

Scheme I





10

PHARMACEUTICAL COMPOSITIONS OF THE PRESENT INVENTION

The present invention also relates to a pharmaceutical composition comprising:

- (i) an effective amount of the compound of formula I; and
- (ii) a pharmaceutically acceptable carrier.

15

Examples of the compound of formula I are set forth above.

METHODS OF THE PRESENT INVENTION

20

METHOD OF INHIBITING NAALADASE ENZYME ACTIVITY

The present invention further relates to a method of inhibiting NAALADase enzyme activity in an animal, comprising administering an effective amount of the compound of formula I to said animal.

25

METHOD OF TREATING GLUTAMATE ABNORMALITY

Although not limited to any one particular theory,
it is believed that the compounds of the present
invention modulate levels of glutamate by acting on a
5 storage form of glutamate which is hypothesized to be
upstream from the effects mediated by the NMDA receptor.

Accordingly, the present invention further relates
to a method of treating a glutamate abnormality in an
animal, comprising administering an effective amount of
10 a prodrug of the compound of formula I to said animal.

The glutamate abnormality may be any disease,
disorder or condition in which glutamate is implicated,
including pathological conditions involving elevated
levels of glutamate. Examples of glutamate abnormalities
15 include without limitation epilepsy, stroke, Alzheimer's
disease, Parkinson's Disease, Amyotrophic Lateral
Sclerosis (ALS), Huntington's Disease, schizophrenia,
chronic pain, ischemia, peripheral neuropathy, traumatic
brain injury and physical damage to the spinal cord. In
20 a preferred embodiment, the glutamate abnormality is
selected from the group consisting of ischemia, stroke,
Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS)
and spinal cord injury.

25

METHOD OF TREATING COMPULSIVE DISORDER

The inventors have unexpectedly found that the compounds of the present invention are effective in treating glutamate-related compulsive disorders.

5 Accordingly, the present invention also relates to a method of treating a compulsive disorder, comprising administering an effective amount of the compound of formula I to a patient in need thereof.

10 The compulsive disorder may be any disorder characterized by irresistible impulsive behavior. Examples of compulsive disorders treatable by the methods of the present invention include drug dependence, eating disorders, pathological gambling, ADD and Tourette's syndrome.

15 Preferably, the compulsive disorder is drug dependence. Commonly used drugs with potential for dependence include CNS depressants (opioids, synthetic narcotics, barbiturates, glutethimide, methyprylon, ethchlorvynol, methaqualone, alcohol); anxiolytics
20 (diazepam, chlordiazepoxide, alprazolam, oxazepam, temazepam); stimulants (amphetamine, methamphetamine, cocaine); and hallucinogens (LSD, mescaline, peyote, marijuana).

25 More preferably, the drug dependence is alcohol, nicotine, heroin or cocaine dependence.

METHOD OF EFFECTING NEURONAL ACTIVITY

The inventors have also discovered that inhibition of NAALADase promotes nerve regeneration and myelin formation.

5 Accordingly, the present invention further relates to a method of effecting a neuronal activity in an animal, comprising administering an effective amount of the compound of formula I to said animal.

10 The neuronal activity that is effected by the method of the present invention may be selected from the group consisting of: stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration and treatment of a neurological disorder.

15 Examples of a neurological disorder that is treatable by the method of the present invention include without limitation: trigeminal neuralgia; glossopharyngeal neuralgia; Bell's Palsy; myasthenia gravis; muscular dystrophy; amyotrophic lateral sclerosis; progressive muscular atrophy; progressive
20 bulbar inherited muscular atrophy; herniated, ruptured or prolapsed invertebrate disk syndromes; cervical spondylosis; plexus disorders; thoracic outlet destruction syndromes; peripheral neuropathies such as those caused by lead, dapsone, ticks, porphyria, or
25 Guillain-Barré syndrome; Alzheimer's disease; and Parkinson's disease.

The method of the present invention is particularly useful for treating a neurological disorder selected from the group consisting of: peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke associated with brain damage, demyelinating diseases and neurological disorders relating to neurodegeneration. Examples of demyelinating diseases include multiple sclerosis. Examples of neurological disorders relating to neurodegeneration include Alzheimer's Disease, Parkinson's Disease, and amyotrophic lateral sclerosis (ALS).

METHOD OF TREATING PROSTATE DISEASE

Additionally, the present invention relates to a method of treating a prostate disease in an animal, comprising administering an effective amount of the compound of formula I to said animal.

In a preferred embodiment, said prostate disease is prostate cancer or benign prostatic hyperplasia.

METHOD OF TREATING CANCER

In addition to prostate cancer, other forms of cancer that may be treated with the compounds of the present invention include without limitation: ACTH-producing tumors, acute lymphocytic leukemia, acute

nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervix cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head & neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and/or non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovary cancer, ovary (germ cell) cancer, pancreatic cancer, penis cancer, retinoblastoma, skin cancer, soft-tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, cancer of the uterus, vaginal cancer, cancer of the vulva and Wilm's tumor.

The compounds of the present invention are particularly useful in treating cancer of tissues where NAALADase enzymes reside. Such tissues include the prostate as well as the brain, kidney and testis.

ROUTE OF ADMINISTRATION

In the methods of the present invention, the compounds may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally,

vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes
5 subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal or intracranial injection and infusion techniques. Invasive techniques are preferred, particularly direct administration to damaged neuronal
10 tissue.

To be effective therapeutically as central nervous system targets, the compounds of the present invention should readily penetrate the blood-brain barrier when peripherally administered. Compounds which cannot
15 penetrate the blood-brain barrier can be effectively administered by an intraventricular route.

The compounds may also be administered in the form of sterile injectable preparations, for example, as sterile injectable aqueous or oleaginous suspensions.
20 These suspensions can be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparations may also be sterile injectable solutions or suspensions in non-toxic parenterally-
25 acceptable diluents or solvents, for example, as solutions in 1,3-butanediol. Among the acceptable

vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils are conventionally employed as solvents or suspending mediums. For this purpose, any bland fixed oil such as a synthetic mono- or di-glyceride may be employed. Fatty acids such as oleic acid and its glyceride derivatives, including olive oil and castor oil, especially in their polyoxyethylated forms, are useful in the preparation of injectables. These oil solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

Additionally, the compounds may be administered orally in the form of capsules, tablets, aqueous suspensions or solutions. Tablets may contain carriers such as lactose and corn starch, and/or lubricating agents such as magnesium stearate. Capsules may contain diluents including lactose and dried corn starch. Aqueous suspensions may contain emulsifying and suspending agents combined with the active ingredient. The oral dosage forms may further contain sweetening and/or flavoring and/or coloring agents.

The compounds may further be administered rectally in the form of suppositories. These compositions can be prepared by mixing the drug with suitable non-irritating excipients which are solid at room temperature, but liquid at rectal temperature such that they will melt in

the rectum to release the drug. Such excipients include cocoa butter, beeswax and polyethylene glycols.

Moreover, the compounds may be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin or the lower intestinal tract.

For topical application to the eye, or ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline or, preferably, as a solution in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, the compounds may be formulated into ointments, such as petrolatum.

For topical application to the skin, the compounds can be formulated into suitable ointments containing the compounds suspended or dissolved in, for example, mixtures with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated into suitable lotions or creams containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax,

cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application to the lower intestinal tract can be effected in rectal suppository formulations (see
5 above) or in suitable enema formulations.

The compounds of the present invention may be administered by a single dose, multiple discrete doses or continuous infusion. Since the compounds are small, easily diffusible and relatively stable, they are well
10 suited to continuous infusion. Pump means, particularly subcutaneous pump means, are preferred for continuous infusion.

DOSAGE

15 Dose levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels being about 0.1 mg to about 1,000 mg. The specific dose level for any particular patient will vary
20 depending upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the rate of excretion; drug combination; the severity of the particular disease being
25 treated; and the form of administration. Typically, in vitro dosage-effect results provide useful guidance on

the proper doses for patient administration. Studies in animal models are also helpful. The considerations for determining the proper dose levels are well known in the art.

5 In a preferred embodiment, the compounds are administered in lyophilized form. In this case, 1 to 100 mg of a compound of the present invention may be lyophilized in individual vials, together with a carrier and a buffer, such as mannitol and sodium phosphate. The
10 compound may be reconstituted in the vials with bacteriostatic water before administration.

 In treating global ischemia, the compounds of the present invention are preferably administered orally, rectally, parenterally or topically at least 1 to 6 times
15 daily, and may follow an initial bolus dose of higher concentration.

 The compounds of the present invention may be administered in combination with one or more therapeutic agents, including chemo-therapeutic agents. TABLE I
20 provides known median dosages for selected chemotherapeutic agents. Specific dose levels for these agents and other therapeutic agents will depend upon considerations such as those identified above for the compounds of the present invention.

TABLE I

	CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
	Asparaginase	10,000 units
	Bleomycin Sulfate	15 units
5	Carboplatin	50-450 mg
	Carmustine	100 mg
	Cisplatin	10-50 mg
	Cladribine	10 mg
10	Cyclophosphamide (lyophilized)	100 mg-2 gm
	Cyclophosphamide (non-lyophilized)	100 mg-2 gm
	Cytarabine (lyophilized powder)	100 mg-2 gm
15	Dacarbazine	100 mg-200 mg
	Dactinomycin	0.5 mg
	Daunorubicin	20 mg
	Diethylstilbestrol	250 mg
	Doxorubicin	10-150 mg
20	Etidronate	300 mg
	Etoposide	100 mg
	Floxuridine	500 mg
	Fludarabine Phosphate	50 mg
	Fluorouracil	500 mg-5 gm
25	Goserelin	3.6 mg
	Granisetron Hydrochloride	1 mg
	Idarubicin	5-10 mg
	Ifosfamide	1-3 gm
30	Leucovorin Calcium	50-350 mg
	Leuprolide	3.75-7.5 mg
	Mechlorethamine	10 mg

	CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
	Medroxyprogesterone	1 gm
	Melphalan	50 gm
	Methotrexate	20 mg-1 gm
	Mitomycin	5-40 mg
5	Mitoxantrone	20-30 mg
	Ondansetron Hydrochloride	40 mg
	Paclitaxel	30 mg
	Pamidronate Disodium	30-*90 mg
10	Pegaspargase	750 units
	Plicamycin	2,500 mcgm
	Streptozocin	1 gm
	Thiotepa	15 mg
	Teniposide	50 mg
15	Vinblastine	10 mg
	Vincristine	1-5 mg
	Aldesleukin	22 million units
	Epoetin Alfa	2,000-10,000 units
	Filgrastim	300-480 mcgm
20	Immune Globulin	500 mg-10 gm
	Interferon Alpha-2a	3-36 million units
	Interferon Alpha-2b	3-50 million units
	Levamisole	50 mg
	Octreotide	1,000-5,000 mcgm
25	Sargramostim	250-500 mcgm

ADMINISTRATION REGIMEN

For the methods of the present invention, any administration regimen regulating the timing and sequence of drug delivery can be used and repeated as necessary to

effect treatment. Such regimen may include pretreatment and/or co-administration with additional therapeutic agents.

5 To maximize protection of nervous tissue from nervous insult, the compounds should be administered to the affected cells as soon as possible. In situations where nervous insult is anticipated, the compounds should be administered before the expected nervous insult. Such situations of increased likelihood of nervous insult include surgery (cartoid endarterectomy, cardiac, 10 vascular, aortic, orthopedic); endovascular procedures such as arterial catheterization (cartoid, vertebral, aortic, cardia, renal, spinal, Adamkiewicz); injections of embolic agents; coils or balloons for hemostasis; 15 interruptions of vascularity for treatment of brain lesions; and predisposing medical conditions such as crescendo transient ischemic attacks, emboli and sequential strokes. Where pretreatment for stroke or ischemia is impossible or impracticable, it is important 20 to get the compounds to the affected cells as soon as possible during or after the event. In the time period between strokes, diagnosis and treatment procedures should be minimized to save the cells from further damage and death.

25 For patients with prostate cancer that is neither advanced nor metastatic, the compounds of the present

invention may be administered (i) prior to surgery or radiation treatment to reduce the risk of metastasis; (ii) during surgery or in conjunction with radiation treatment; and/or (iii) after surgery or radiation therapy to reduce the risk of recurrence and to inhibit the growth of any residual tumorous cells.

For patients with advanced or metastatic prostate cancer, the compounds of the present invention may be administered as a continuous supplement to, or as a replacement for, hormonal ablation in order to slow tumor cell growth in both the untreated primary tumor and the existing metastatic lesions.

The methods of the present invention are particularly useful where shed cells could not be removed by surgical intervention. After post-surgical recovery, the methods of the present invention would be effective in reducing the chances of recurrence of a tumor engendered by such shed cells.

COMBINATION WITH OTHER TREATMENTS

a. Nervous Insult

In methods of treating nervous insult (particularly acute ischemic stroke and global ischemia caused by drowning and head trauma), the compounds of the present invention can be co-administered with one or more therapeutic agents, preferably agents which can reduce

the risk of stroke (such as aspirin), and more preferably agents which can reduce the risk of a second ischemic event (such as ticlopidine).

5 The compounds of the present invention can be co-administered with one or more therapeutic agents either (i) together in a single formulation, or (ii) separately in individual formulations designed for optimal release rates of their respective active agent. Each formulation may contain from about 0.01% to about 99.99% by weight, 10 preferably from about 3.5% to about 60% by weight, of a compound of the present invention, as well as one or more pharmaceutical excipients, such as wetting, emulsifying and pH buffering agents.

b. Prostate Disease

15 **(i) Surgery and Radiation Treatment**

In general, surgery and radiation treatment are employed as potentially curative therapies for patients with localized prostate cancer who are under 70 years of age and are expected to live at least 10 more years.

20 Approximately 70% of newly diagnosed prostate cancer patients fall into this category. Approximately 90% of these patients (65% of total patients) undergo surgery, while approximately 10% of these patients (7% of total patients) undergo radiation treatment.

25 Histopathological examination of surgical specimens reveals that approximately 63% of patients undergoing

surgery (40% of total patients) have locally extensive tumors or regional (lymph node) metastasis that was undetected at initial diagnosis. These patients are at a significantly greater risk of recurrence.

5 Approximately 40% of these patients will actually develop recurrence within five years after surgery. Results after radiation treatment are even less encouraging. Approximately 80% of patients who have undergone radiation treatment as their primary therapy have disease

10 persistence or develop recurrence or metastasis within five years after treatment.

Currently, most prostate cancer patients undergoing surgery and radiation treatment do not receive any immediate follow-up therapy. Rather, they are monitored

15 frequently for elevated Prostate Specific Antigen ("PSA"), which is the primary indicator of recurrence or metastasis.

Based on the above statistics, there is considerable opportunity to use the present invention in conjunction

20 with surgery and/or radiation treatment.

(ii) Hormonal Therapy

Hormonal ablation is the most effective palliative treatment for the 10% of patients with metastatic prostate cancer. Hormonal ablation by medication and/or

25 orchiectomy is used to block hormones that promote further growth and metastasis of prostate cancer. With

time, both the primary and metastatic tumors of virtually all of these patients become hormone-independent and resistant to therapy. Approximately 50% of patients with metastatic cancer die within three years after initial
5 diagnosis, and 75% of such patients die within five years after diagnosis. Continuous supplementation with the compounds of the present invention may be used to prevent or reverse this potentially metastasis-permissive state.

(iii) Chemotherapy

10 While chemotherapy has been successful in treating some forms of cancer, it has shown slight therapeutic value in treating prostate cancer where it is generally reserved as a last resort. Accordingly, the opportunity to treat prostate cancer by combining chemotherapy with
15 the methods of the present invention will be rare. When combined, however, such treatments should be more effective than chemotherapy alone in controlling prostate cancer.

(iv) Immunotherapy

20 The compounds of the present invention may also be used in combination with monoclonal antibodies to treat prostate cancer. Such combined treatment is particularly effective for patients with pelvic lymph node involvement, of which only 34% survive after 5 years. An
25 example of such monoclonal antibodies is cell membrane-specific anti-prostate antibody.

The present invention may also be used with immunotherapies based on polyclonal or monoclonal antibody-derived reagents. Monoclonal antibody-derived reagents are preferred. These reagents are well known in the art, and include radiolabelled monoclonal antibodies such as monoclonal antibodies conjugated with strontium-89.

(v) Cryotherapy

The methods of the present invention may also be used in conjunction with cryotherapy for treatment of prostate cancer.

In Vivo Toxicity of NAALADase Inhibitors

To examine the toxicological effect of NAALADase inhibition *in vivo*, a group of mice were injected with 2-(phosphonomethyl)pentanedioic acid, a NAALADase inhibitor of high activity, in doses of 1, 5, 10, 30, 100, 300 and 500 mg/kg body weight. The mice were subsequently observed two times per day for 5 consecutive days. The survival rate at each dose level is provided below in TABLE I. The results show that the NAALADase inhibitor is non-toxic to mice, suggesting that it would be similarly non-toxic to humans when administered at therapeutically effective amounts.

TABLE I

TOXICOLOGICAL EFFECTS OF NAALADASE INHIBITORS							
Dose (mg/kg)	1	5	10	30	100	300	500
Survival Rate After 5 days (%)	100	100	100	100	100	100	66.7

In Vitro Inhibition of NAALADase Activity

Various compounds of formula I were tested for *in vitro* inhibition of NAALADase activity. The results are provided below in Table II.

TABLE II
IN VITRO INHIBITION OF NAALADASE ACTIVITY

5	Compound	K_i (nM)
10		740.0000
15		198.5000
20		4250.0000

TABLE II (continued)
IN VITRO INHIBITION OF NAALADASE ACTIVITY

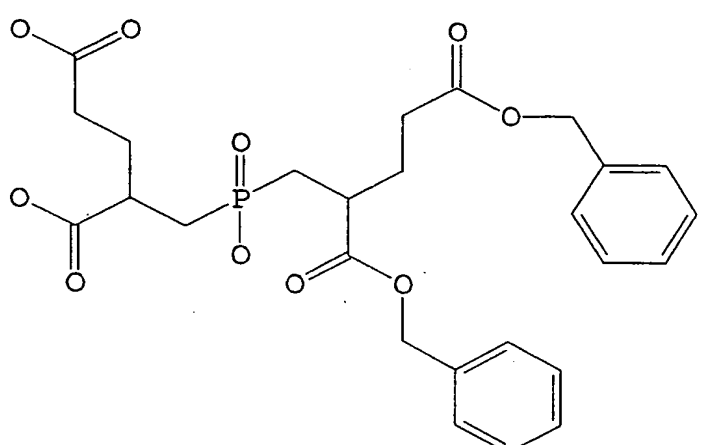
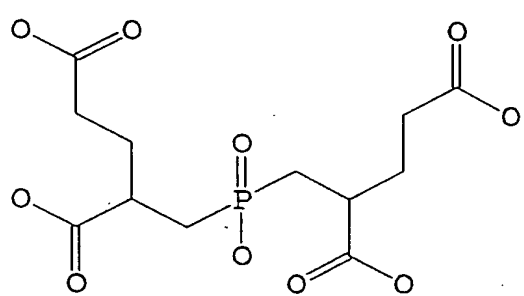
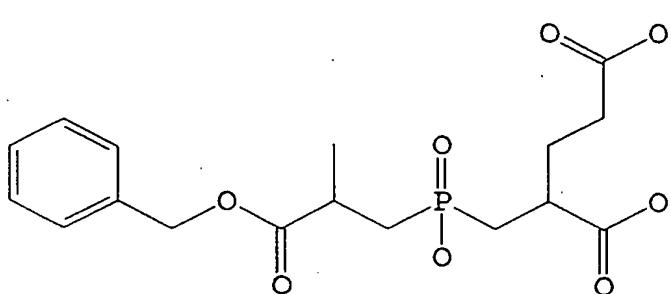
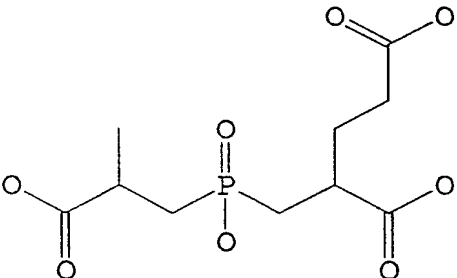
	Compound	K_i (nM)
5		
10		12.6667
15		
20		0.5700
25		
		95.0000

TABLE II (continued)
IN VITRO INHIBITION OF NAALADASE ACTIVITY

Compound	K_i (nM)
	1.5000

Protocol for Assaying In Vitro Inhibition of NAALADase

Activity

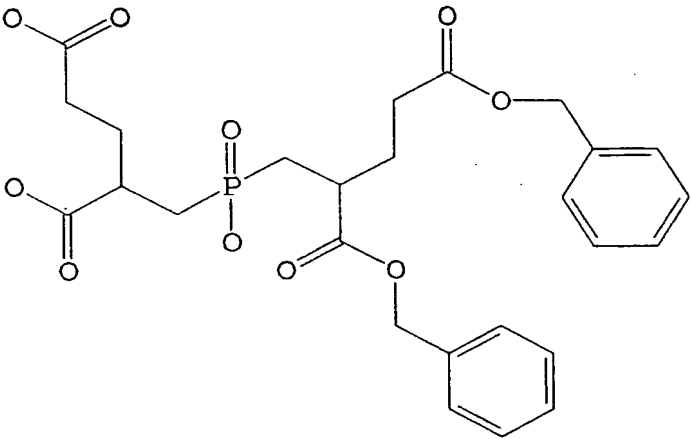
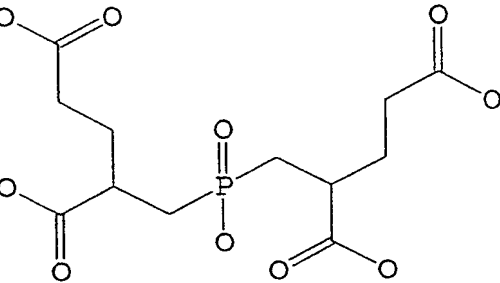
The amount of [3 H]Glu liberated from [3 H]NAAG in 50 mM Tris-Cl buffer was measured for 15 minutes at 37° C using 30-50 μ g of synaptosomal protein. Substrate and product were resolved by anion-exchange liquid chromatography. Duplicate assays were performed so that no more than 20% of the NAAG was digested, representing the linear range of peptidase activity. Quisqualate (100 μ M) was included in parallel assay tubes to confirm the specificity of the measurements.

In Vitro Assay of NAALADase Inhibitors on Ischemia

To examine the *in vitro* effect of NAALADase inhibitors on ischemia, cortical cell cultures were treated with compounds of formula I during an ischemic insult (potassium cyanide and 2-deoxyglucose) and for one hour thereafter (for experimental details, see Vornov et al., *J. Neurochem*, Vol. 65, No. 4, pp. 1681-1691 (1995)).

The neuroprotective effect of each tested compound is provided below in TABLE III(a). Neuroprotective effect is expressed as EC_{50} , the concentration which is required to cause a 50% reduction in glutamate toxicity following an ischemic insult.

TABLE III(a)

Compound	EC ₅₀ (nM)
<div data-bbox="196 436 245 464">5</div> <div data-bbox="196 730 245 758">10</div> 	0.0110
<div data-bbox="196 1031 245 1058">15</div> <div data-bbox="196 1331 245 1358">20</div> 	0.0360

25

The dose-response of this effect, as measured by the % toxicity at different concentrations of NAALADase inhibitor 2-(phosphonomethyl)pentanedioic acid, is provided below in TABLE III(b) and graphically presented in FIG. 1.

TABLE III(b)

<u>Dose</u>	<u>% Toxicity</u>
Control	100.00 \pm 9.0 (n = 5)
100 pM	66.57 \pm 4.38 (n = 5)
1 nM	42.31 \pm 9.34 (n = 5)
10 nM	33.08 \pm 9.62 (n = 5)
100 nM	30.23 \pm 9.43 (n = 5)
1 μ M	8.56 \pm 8.22 (n = 5)

The results show that toxicity decreased as the concentration of 2-(phosphonomethyl)pentanedioic acid increased, suggesting that NAALADase inhibitors would be effective in treating ischemia or neuronal damage caused by ischemia.

The methods for this assay are described in detail below. Specifically, cell cultures were exposed to potassium cyanide and 2-deoxyglucose (2-DG) (10 mM) and analyzed for release of lactate dehydrogenase (LDH).

In Vitro Toxicity of NAAG

To examine the *in vitro* toxicity of NAAG, cortical cell cultures were treated with NAAG (in concentrations ranging from 3 μ M to 3 mM) for 20 minutes. The toxicity measurement for each concentration of NAAG is provided below in TABLE IV and graphically presented in FIG. 2.

TABLE IV

	<u>Dose of NAAG</u>	<u>% Toxicity</u>	
10	3 μ M	3.51	(n = 1)
	10 μ M	4.30 \pm 3.12	(n = 3)
	30 μ M	11.40 \pm 6.17	(n = 3)
	100 μ M	12.66 \pm 5.50	(n = 3)
	300 μ M	13.50 \pm 4.0	(n = 3)
15	1 mM	21.46 \pm 4.20	(n = 3)
	3 mM	45.11 \pm 4.96	(n = 3)

The results show that toxicity increased as the concentration of NAAG increased. The toxicity is attributed to the release of glutamate by NAAG when cleaved by NAALADase.

In Vitro Assay of NAALADase Inhibitors on Toxicity of
NAAG

To examine the effect of NAALADase inhibitors on *in vitro* toxicity of NAAG, cortical cell cultures were

treated with 2-(phosphonomethyl)pentanedioic acid (1 μ M) during exposure to NAAG and for one hour thereafter. The toxicity measurement for each concentration of NAAG is provided below in TABLE V and graphically presented in FIG. 3.

TABLE V

	<u>Dose of NAAG</u>	<u>% Toxicity</u>
	3 μ M	-4.71 (n = 1)
10	10 μ M	-3.08 \pm 0.81 (n = 3)
	30 μ M	-4.81 \pm 1.13 (n = 3)
	100 μ M	-2.87 \pm 0.78 (n = 3)
	300 μ M	-2.09 \pm 0.48 (n = 3)
	1 mM	0.26 \pm 1.11 (n = 3)
15	3 mM	16.83 \pm 8.76 (n = 3)

When compared to the results of FIG.2/TABLE IV, the results of FIG.3/TABLE V show that toxicity decreased considerably after treatment with the NAALADase inhibitor, suggesting that it would be effective in treating glutamate abnormalities.

In Vitro Assay of NAALADASE Inhibitors on Ischemia at
Different Times of Administration

To examine the effect of NAALADase inhibitors on *in vitro* ischemic toxicity at different times of

administration, cortical cell cultures were treated with 2-(phosphonomethyl)pentanedioic acid (i) during an ischemic insult and for one hour thereafter (exposure and recovery); (ii) for one hour following ischemic insult (recovery only); and (iii) for one hour beginning 30 minutes after ischemic insult (delayed 30 minutes). The toxicity measurement for each time of administration is provided below in TABLE VI and graphically presented in FIG. 4.

TABLE VI

<u>Time of Administration</u> <u>Relative to Ischemic Insult</u>	<u>% Toxicity</u>
Control	100.00%
Exposure & Recovery	2.54%
Recovery Only	9.03%
Delayed 30 Minutes	31.49%

The results show that significant neuronal protection is achieved when NAALADase inhibitors are administered during exposure and recovery from an ischemic insult, and even after a 30 minute delay following the ischemic insult.

Protocol for In Vitro Toxicity Assaya. Cell Culture

Dissociated cortical cell cultures are prepared using the papain-dissociation method of Heuttner and Baughman (1986) as modified by Murphy and Baraban (1990). See TABLE VII for the Dissociated Culture Protocol as used herein. Fetuses of embryonic day 17 are removed from timed pregnancy rats (Harlan Sprague Dawley). The cortex is rapidly dissected out in Dulbecco's phosphate-buffered saline, stripped of meninges, and incubated in a papain solution for 15 minutes at 37° C. The tissue is then mechanically triturated and pelleted at 500 g (1000-2000 rpm on swinging bucket Beckman). The pellet is resuspended in a DNAase solution, triturated with a 10 ml pipette x15-20, layered over a "10 x 10" solution containing albumin and trypsin inhibitor (see TABLE VII for an example of a "10 x 10" solution), repelleted, and resuspended in a plating medium containing 10% fetal bovine serum (HyClone A-1111-L), 5% heat-inactivated Equine serum (HyClone A-3311-L), and 84% modified Earle's basal medium (MEM) (Gibco 51200-020) with high glucose (4.5 g/L), and 1 g/L NaHCO₃. Each 24-well plate is pretreated with poly-D-lysine (0.5 ml/well of 10 µg/ml) for 1 h and rinsed with water before plating. Cultures are plated at 2.5 x 10⁶ cells/ml with each well of a 24 well plate receiving 500µl/well. Alternatively, 35 mm

dishes can be plated at 2 ml/dish, 6 well plates at 2 ml/well, or 12 well plates at 1 ml/well. After plating, 50% of the medium is changed every 3-4 days with growth serum containing 5% heat-inactivated Equine serum (HyClone A-3311-L), 95% modified Earle's basal medium (MEM) (Gibco 51200-020), and 1% L-Glutamine (Gibco 25030-081). Experiments are performed after 21 days in cultures. Cultures are maintained in a 5% CO₂ atmosphere at 37° C. These methodologies are described in further detail below in the TABLE VII.

TABLE VII

DISSOCIATED CULTURE PROTOCOL	
I. PREPARE SOLUTIONS	
Stocks/Solutions	
5	<u>DNAase Stock, 1 ml</u> <u>(100x)</u> 5 mg DNAase I (Worthington LS002004); 1 ml dissoc. EBSS; freeze as 50 μ l aliquots.
10	<u>Dulbecco's PBS, 500 ml</u> 4 gm NaCl (J.T. Baker 3624-01); 1.06 gm $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (Fisher S373-3); 100 mg KCl (Fisher P217- 500); 100 mg KH_2PO_4 (Sigma P- 0662); 500 ml dH_2O ; adjust pH to 7.4 and sterile filter.
15	<u>Dissociated EBSS, 500</u> <u>ml</u> 1.1 gm NaHCO_3 ; 50 ml EBSS stock (Gibco 14050-025); 450 ml dH_2O ; sterile filter.
	<u>EDTA Stock, 10 ml</u> 184.2 mg EDTA sodium salt (Sigma ED4S); 10 ml dH_2O ; sterile filter.

5

10

DISSOCIATED CULTURE PROTOCOL	
<u>10 and 10 Stock, 10 ml</u> 100 mg BSA (Sigma A-4919); 100 mg Trypsin Inhibitor from Egg White (Sigma T-2011); 10 ml dissoc. EBSS; sterile filter.	<u>Poly-D-Lysine Stock, 5 ml</u> 5 mg Poly-D-Lysine, 100-150 K (Sigma P-6407); 5 ml sterile water; keep frozen.
Media	

DISSOCIATED CULTURE PROTOCOL

<u>Dissociated growth,</u>	<u>Plating media, 300 ml</u>
500 ml	
500 ml MEM (Gibco	250 ml MEM containing
51200-020) containing	glucose and sodium
5 glucose and NaHCO ₃	bicarbonate (2.25 gm
(2.25 gm glucose and	glucose and 0.5 gm NaHCO ₃
0.5 gm NaHCO ₃);	in 500 ml Gibco MEM 51200-
25 ml heat-	020);
inactivated Equine	30 ml Fetal Bovine Serum
Serum (HyClone A-	(HyClone A-1111-L).
3311-L);	
5 ml L-Glutamine (200	
mM, 100x stock, Gibco	
25030-081);	
sterile filter.	
15 ml heat-	
inactivated Equine	
Serum (HyClone A-	
3311-L);	
3 ml L-Glutamine (200	
mM, 100x stock, Gibco	
25030-081); (Gibco	
15140-015);	
1 ml Penicillin-	
Streptomycin stock.	

DISSOCIATED CULTURE PROTOCOL

For papain dissociation:

4 mg Cysteine (C-
8277);
25 ml dissoc. EBSS;
250 μ l Papain stock
(Worthington
LS003126);
place in 37°C
waterbath until
clear.

For DNAase treatment:

DNAase, 5 ml

4.5 ml dissoc. EBSS;
500 μ l "10 and 10" stock;
50 μ l DNAase stock.

"10 and 10", 5 ml

4.5 ml of EBSS;
500 μ l "10 and 10" stock.

II. COAT DISHES

Use poly-d-lysine stock at 1:100 dilution to coat
24-well plates (0.5 ml/well) or at 1:10 dilution
to coat 35 mm glass cover slips (1.0
ml/coverslip).
Leave until end of dissection.

DISSOCIATED CULTURE PROTOCOL**III. DISSECT TISSUE**

5 Use Harlan Sprague-Dawley timed pregnancy rats,
ordered to arrive at E-17.

Decapitate, spray abdomen down with 70% EtOH.

Remove uterus through midline incision and place
in sterile dPBS.

10 Remove brains from embryos, leaving them in dPBS.

Brain removal: Penetrate skull and skin with fine
forceps at lambda. Pull back to open posterior
fossa. Then move forceps anteriorly to separate
sagittal suture. Brain can be removed by scooping
15 back from olfactory bulbs under the brain.

Move brains to fresh dPBS; subsequently, dissect
away from cortex.

DISSOCIATED CULTURE PROTOCOL**IV. PAPAIN DISSOCIATION**

____ Transfer cortices equally to two 15 ml tubes containing sterile papain solution, maintained at 37° C.

Triturate x1 with sterile 10 ml pipette.

Incubate only for 15 minutes at 37° C.

Spin at 500 G for 5 minutes (1000-2000 RPM on swinging bucket Beckman).

V. DNAase TREATMENT

____ Remove supernatant and any DNA gel layer from cell pellet (or pick up and remove pellet with pipette).

Move cell pellet to DNAase solution.

Triturate with 10 ml pipette, x15-20.

Layer cell suspension over the "10 and 10" solution by pipetting it against the side of the tubes.

Spin again at 500 G for 5 minutes (cells will spin into "10 and 10" layer).

Wash tube sides with plating media without disturbing pellet.

Pipette off the media wash and repeat the wash.

DISSOCIATED CULTURE PROTOCOL

VI. PLATE

___Add about 4.5 ml plating media to each pellet
for 5 ml volume.

5 Re-suspend with 10 ml pipette.

Pool cells into a single tube.

Quickly add 10 μ l of the suspended cells to a
hemocytometer so that they do not settle.

10 Count cells per large square, corresponding to 10
million cells/ml.

Put re-suspended cells into a larger container so
that they number 2.5 million cells/ml.

Triturate to homogeneity.

15 Finish coating plates:

Aspirate or dump Lysine;

Wash x1 with sterile water and dump.

20 Add plating media, with cells, to the plates as
follows:

35 mm dishes 2 ml/dish;

6 well plate 2 ml/well;

12 well plate 1 ml/well;

24 well plate 500 μ l/well.

DISSOCIATED CULTURE PROTOCOL**VII. FEED**

Cultures are usually made on Thursdays.
Start feeding twice a week; beginning the
following Monday, feedings on Mondays and Fridays.
Remove 50% of volume and replace with fresh growth
media.

**b. Ischemic Insult using potassium cyanide and
2-deoxyglucose**

Twenty-one to twenty-four days following the initial
cortical cell plating, the experiment is performed. The
cultures are washed three times in HEPES buffered saline
solution containing no phosphate. The cultures are then
exposed to potassium cyanide (KCN) (5 mM) and 2-
deoxyglucose (2-DG) (10 mM) for 20 minutes at 37° C.
These concentrations were shown previously to induce
maximal toxicity (Vornov et al., *J. Neurochem*, Vol. 65,
No. 4, pp. 1681-1691 (1995)). At the end of 24 hours,
the cultures are analyzed for release of the cytosolic
enzyme lactate dehydrogenase (LDH), a standard measure of
cell lysis. LDH measurements are performed according to
the method of Koh and Choi, *J. Neuroscience Methods*
(1987).

c. NAAG Induced Neurotoxicity

Cultures are assessed microscopically and those with uniform neuronal densities are used in the NAAG neurotoxicity trials.

5 At the time of the experiment, the cultures are washed once in HEPES-buffered saline solution (HBSS; NaCl 143.4 mM, HEPES 5 mM, KCl 5.4 mM, MgSO₄ 1.2 mM, NaH₂PO₄ 1.2 mM, CaCl₂ 2.0 mM, D-glucose 10 mM) (Vornov et al., 1995) and then exposed to various concentrations of NAAG
10 for 20 minutes at 37° C. NAAG concentrations range from 3 µM to 3 mM, and include 3 µM, 10 µM, 30 µM, 100 µM, 300 µM, 1 mM, and 3 mM. At the end of exposure, the cells are washed once with HEPES buffered saline solution and then replaced with serum free modified Earle's basal
15 medium. The cultures are then returned to the CO₂ incubator for 24 hour recovery.

d. Lactate Dehydrogenase Assay

Release of the cytosolic enzyme lactate dehydrogenase (LDH), a standard measure of cell lysis, is
20 used to quantify injury (Koh and Choi, 1987). LDH-activity measurements are normalized to control for variability between culture preparations (Koh and Choi, 1987). Each independent experiment contains a control condition in which no NAALADase inhibitors are added; a
25 small amount of LDH activity is found in these controls. This control measurement is subtracted from each

experimental point. These values are normalized within each experiment as a percentage of the injury caused by NAAG/ischemia. Only main effects of NAALADase inhibitors are considered; interactions between dose and condition are not examined statistically.

A measurement of the potency of each compound tested is made by measuring the percentage of LDH release into the growth media after exposure to NAAG/ischemia plus NAALADase inhibitor or NAAG/ischemia plus saline (control). Since high concentrations of glutamate may be toxic to cells in certain circumstances, measurement of glutamate toxicity is observed using LDH as a standard measurement technique.

In Vivo Assay of NAALADase Inhibitors on Cortical Injury following MCAO in SHRSP Rats

To examine the effect of NAALADase inhibitors on cortical injury *in vivo*, the infarct volume was measured in SHRSP rats which had sustained middle cerebral artery occlusion (MCAO) and had subsequently been treated with (i) saline; (ii) 10 mg/kg of 2-(phosphonomethyl)-pentanedioic acid followed by 2 mg/kg/hr of 2-(phosphonomethyl)pentanedioic acid for 1 hour; or (iii) 100 mg/kg of 2-(phosphonomethyl)pentanedioic acid followed by 20 mg/kg/hr of 2-(phosphonomethyl)-pentanedioic acid for one hour.

The cortical injury volume for each group of rats is provided below in TABLE VIII and graphically presented in FIG. 5.

TABLE VIII

5	Cortical Injury Volume (mm³)\pmS.E.M.	
	Control	184.62 \pm 33.52 (n = 10)
	10 mg/kg	135.00 \pm 32.18 (n = 10)
	100 mg/kg	65.23 \pm 32.18 (n = 10)
10	Cortical Injury Volume (% injury)\pmS.E.M.	
	Control	34.44 \pm 6.53 (n = 10)
	10 mg/kg ³	29.14 \pm 7.68 (n = 10)
	100 mg/kg	13.98 \pm 6.64 (n = 10)
15	Cortical Protection (% protection)	
	Control	0%
	10 mg/kg	27%
	100 mg/kg	65%

The results show that cortical injury volume decreased and cortical protection increased as the amount of NAALADase inhibitor increased, further supporting the neuroprotective effect of the NAALADase inhibitor.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Cortical Injury

A colony of SHRSP rats is bred at Johns Hopkins School of Medicine from three pairs of male and female rats obtained from the National Institutes of Health (Laboratory, Sciences Section, Veterinary Resources Program, National Center for Research Resources, Bethesda, MD). All rats are kept in a virus-free environment and maintained on regular diet (NIH 31, Zeigler Bros, Inc.) with water ad libitum. All groups of rats are allowed to eat and drink water until the morning of the experiment.

Transient occlusion of the middle cerebral artery (MCA) is induced by advancing a 4-0 surgical nylon suture into the internal carotid artery (ICA) to block the origin of the MCA (Koizumi, 1986; Longa, 1989; Chen, 1992). The rats are anesthetized with 4% halothane, and maintained with 1.0% to 1.5% halothane in air enriched oxygen using a face mask. Rectal temperature is maintained at $37.0 \pm 0.5^{\circ}\text{C}$ throughout the surgical procedure using a heating lamp. The right femoral artery is cannulated for measuring blood gases (pH, oxygen tension $[\text{PO}_2]$, carbon dioxide tension $[\text{PCO}_2]$) before and during ischemia, for monitoring blood pressure during the surgery. The right common carotid artery (CCA) is exposed through a midline incision; a self-retraining

retractor is positioned between the digastric and mastoid muscles, and the omohyoid muscle is divided. The right external carotid artery (ECA) is dissected and ligated. The occipital artery branch of the ECA is then isolated and coagulated. Next, the right internal carotid artery (ICA) is isolated until the pterygopalatine artery is exposed, and carefully separated from the adjacent vagus nerve. The pterygopalatine artery is ligated with 4-0 silk suture close to its origin.

After the CCA is ligated with 4-0 silk suture, a 4-0 silk suture to prevent bleeding from a puncture site, through which a 2.5 cm length of 4-0 monofilament nylon suture (Ethilon), its tip rounded by heating near a electric cautery, is introduced into the ICA lumen. A 6-0 silk suture is tightened around the intraluminal nylon suture at the bifurcation to prevent bleeding, and the stretched sutures at the CCA and the ICA are released. The nylon suture is then gently advanced as far as 20 mm.

Anesthesia is terminated after 10 minutes of MCA occlusion in both groups, and the rats were awakened 5 minutes thereafter. After 2 hours of ischemia, anesthesia is reanesthetized, and reperfusion is performed by withdrawing the intraluminal nylon suture until the distal tip became visible at the origin of the ICA.

Arterial pH and PaCO_2 , and partial pressure of

oxygen (PaO_2) are measured with a self-calibrating Radiometer electrode system (ABL 3; Copenhagen, Denmark). Hemoglobin and arterial oxygen content are measured with a hemoximeter (Radiometer, Model OSM3; Copenhagen, Denmark). Blood glucose is measured with a glucose analyzer (model 2300A, Yellow Springs Instruments, Yellow Springs, OH).

Each group is exposed to 2 hours of right MCA occlusion and 22 hours of reperfusion. All variables but the rectal temperature are measured at baseline, at 15 minutes and 45 minutes of right MCA occlusion. The rectal temperature is measured at baseline, at 0 and 15 min of MCA occlusion, and at 0 and 22 hours of reperfusion.

In Vivo Assay of NAALADase Inhibitors on Brain Injury following MCAO in Sprague-Dawley Rats

To examine the neuroprotective effect of NAALADase inhibitors on brain injury in vivo, Sprague-Dawley rats were treated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid before and after sustaining a 2 hour transient middle cerebral artery occlusion (MCAO). In the control group ($n = 8$), the rats received an IP injection of saline 30 minutes post-occlusion followed by IV saline infusion at a rate of 0.5 ml/hr. In the drug treated groups, the rats received an IP injection of 2-

(phosphonomethyl)pentanedioic acid at a dose of 100 mg/kg at 20 minutes pre-occlusion (n = 5), 30 minutes post-occlusion (n = 9), 60 minutes post-occlusion (n = 7), or 120 minutes post-occlusion (n = 4), followed by a 20 mg/kg/hr IV infusion for 4 hours (infusion rate = 0.5 ml/hr). There was a 15 minute delay between IP and IV treatments for each rat. Twenty two hours following the reperfusion, the rats were euthanized and their brains were removed. Seven coronal sections (2 mm thick) were taken and stained with 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 20 minutes and then fixed in 10% formalin. The anterior and posterior surface of the most rostral brain section and the posterior surface of each of the other 6 sections were imaged. The quantification of infarct size of each brain was obtained using a computer aided-digital imaging analysis system (LOATS). The brain regions completely lacking TTC-staining were characterized as representative of infarcted tissue. The total infarct volume for each rat was calculated by numeric integration of the respective sequential brain areas.

The total infarct volume for each group of rats is graphically presented in FIG. 6.

Vehicle treated rats exhibited a mean total brain infarct volume of $293 \pm 26 \text{ mm}^3$. Rats treated with 2-(phosphonomethyl)pentanedioic acid either before or after

the ischemic insult exhibited significantly lower mean total brain infarct volumes of $122 \pm 26 \text{ mm}^3$ ($p = 0.003$ vs. vehicle) for 20 minute pre-treatment, $208 \pm 40 \text{ mm}^3$ ($p = 0.2$ vs. vehicle) for 30 minute post-treatment, $125 \pm 57 \text{ mm}^3$ ($p = 0.015$ vs. vehicle) for 60 minute post-treatment, and $133 \pm 35 \text{ mm}^3$ ($p = 0.005$ vs. vehicle) for 120 minute post-treatment. These results indicate that 2-(phosphonomethyl)pentanedioic acid is neuroprotective in rat MCAO model of stroke when administered up to 2 hours post-occlusion.

**Protocol for In Vivo Assay of NAALADase Inhibitors on
Brain Injury**

Male Sprague-Dawley rats (260-320 g) were used. Prior to the experiment, the rats were individually housed and allowed free access to food and water. Each rat received two surgeries: jugular vein cannulation for IV infusion and MCAO. During surgeries, the rat was anesthetized with 2% halothane delivered in oxygen via an inhalation mask. The body temperature was monitored and regulated at normothermic level using a homeothermic heating system. First, a PE-50 polyethylene catheter was inserted into the right jugular vein. One hour later, the rat was reanesthetized for MCAO surgery. The MCAO was achieved using the endovascular suture method described by Long et al., *Stroke*, Vol. 20, pp. 84-91

(1989). Specifically, the right external carotid artery (ECA) was exposed, coagulated and transected. A 3-0 monofilament nylon suture with a blunted tip was introduced into the proximal stump of the ECA via an arteriotomy and advanced 20 mm from the carotid bifurcation until it lodged in the proximal region of the anterior cerebral artery, thereby occluding the origin of the MCA. The rats were allowed to wake up; 2 hours later, the rats were reanesthetized for reperfusion, during which the nylon suture was retracted to the stump of the ECA allowing blood recirculation to the MCA.

In Vivo Assay of NAALADase Inhibitors on Stroke-Induced Rise in Brain Glutamate Levels

To examine the effect of NAALADase inhibitors on hyperglutamatergic disorders *in vivo*, rats with stroke-induced rise in brain glutamate levels were treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid.

The results are graphically presented in FIGS. 7, 8 and 9.

The results show that 2-(phosphonomethyl)-pentanedioic acid treatment (100 mg/kg IP followed by 20 mg/kg/hr IV) significantly attenuated stroke-induced extracellular glutamate increases in the striatum (FIG. 7) as compared to vehicle treated rats ($p < 0.05$), and completely prevented concurrent glutamate changes in the

parietal cortex ($p < 0.01$; FIG. 8). In contrast, there was no significant effect of the stroke itself on glutamate in the frontal cortex and no subsequent difference between the vehicle and 2-(phosphonomethyl)-pentanedioic acid treated groups (FIG. 9). Values are expressed as % baseline where baseline constitutes the mean of three consecutive 20 minute samples preceding stroke. Absolute basal (pretreatment) values for glutamate (mean \pm SEM) in caudate, parietal and frontal cortices were 0.25 ± 0.1 , 1.1 ± 0.3 and 0.6 ± 0.1 μM , respectively, in the vehicle treated rats, and 0.46 ± 0.1 , 2.0 ± 0.7 and 0.9 ± 0.3 μM , respectively, in the 2-(phosphonomethyl)pentanedioic acid treated rats.

Protocol for In Vivo Assay of NAALADase Inhibitors on Stroke-Induced Rise in Brain Glutamate Levels

Male Sprague Dawley rats (270-330 g, $n = 5-6$ per group) were implanted with concentric microdialysis probes similar to previously described procedures (Britton et al., *J. Neurochem.*, Vol. 67, pp. 324-329 (1996)). In brief, under halothane anaesthesia, probes (constructed in-house using Cuprophane capillary membrane; 10K mw cut off; 2 mm dialyzing length) were implanted into the frontal cortex (AP = +3.5; ML = 3; DIV = 3), caudate nucleus (AP = 0; ML = 3; DV = 6.6), and parietal cortex (AP = -2; ML = 5; DV = 3) (coordinates in

mm relative to bregma and dura, respectively), regions believed to represent core and penumbral areas of ischemia-induced injury. Glutamate levels in dialysate were determined using precolumn o-phthaldialdehyde derivatization, followed by HPLC with fluorometric detection.

Approximately 20 hours after probe implantation, the rats were dialyzed with perfusion fluid (125 mM NaCl, 2.5 mM KCl, 1.18 mM MgCl₂ and 1.26 mM CaCl₂) at a rate of 2.5 μ l/min. Following a 60 minute stabilization period, dialysis samples were collected every 20 minutes. After collecting 3 baseline samples, the rats were anaesthetized with halothane and subjected to temporary ischemia using the filament method of MCAO (Britton et al., *Life Sciences*, Vol. 60, No. 20, pp. 1729-1740 (1997)). In brief, the right external carotid artery (ECA) was exposed and its branches coagulated. A 3-0 monofilament nylon suture was introduced into the internal carotid artery via an arteriotomy in the ECA and advanced until it lodged in the proximal region of the anterior cerebral artery, thus occluding the origin of the MCA. The endovascular suture was retracted to allow reperfusion 2 hours after occlusion.

Body temperature was maintained normothermic throughout stroke surgery and reperfusion procedures. The rats were dosed IP with 100 mg/kg 2-

(phosphonomethyl)pentanedioic acid at -20 minute pre-occlusion and IV with 20 mg/kg/hr for 4 hours at the time of occlusion. Dialysis samples were collected every 20 minutes from unanesthetized rats. Following 24 hours of
5 reperfusion, the rats were sacrificed, their brains were removed, and 7 coronal sections (2 mm thick) were taken from the region beginning 1 mm from the frontal pole and ending just rostral to the cortico-cerebellar junction. Analysis of ischemic cerebral damage was achieved using
10 TTC staining and computer assisted image analysis as described by Britton et al. (1997), *supra*.

**In Vivo Assay of NAALADase Inhibitors on Myelin
Formation Following Sciatic Nerve Cryolesion**

15 It was recently demonstrated that NAALADase is down-regulated in glial cells as they start to form myelin and is absent in myelinating Schwann cells. Based on this data, the inventors hypothesized that inhibition of NAALADase may affect the signaling mechanism between
20 axons and Schwann cells and result in increasing myelination. To test this hypothesis, the inventors examined the effect of 2-(phosphonomethyl)pentanedioic acid on nerve regeneration and myelination following cryolesion of the sciatic nerve in male mice.

25 The results are provided below in TABLE IX and graphically presented in FIG. 10(a) and FIG. 10(b).

TABLE IX
IN VIVO EFFECT OF NAALADASE INHIBITORS ON MYELIN
FORMATION FOLLOWING SCIATIC NERVE CRYOLESION

5	2- (phosphonomethyl) - pentanedioic acid	vehicle
10	ratio of # of myelinated axons (drug/vehicle)	1.5
15	# of myelinated lamellae (ave. + SEM)	16.53 ± 0.65 13.77 ± 0.09
15	% increase of myelinated lamellae over vehicle	20%
20	significance by t-test	p < 0.005

25 As detailed in FIG. 10(a) and FIG. 10(b), both light and transmission electron microscopy (TEM) examination of the nerve 3 mm distal to the site of cryolesion

demonstrated a significant increase in the number of myelinated axons (1.5-fold increase) and myelin thickness (20% increase, $p < 0.005$), as compared to nerves in mice treated with vehicle.

5 FIG. 10(a) and FIG. 10(b) show a photomicrograph of this effect. Sections were stained with toluidine blue which stains myelin. Sciatic nerves treated with 2-(phosphonomethyl)-pentanedioic acid containing implants, compared with sciatic nerves treated with vehicle
10 containing implants, exhibited an increase in myelinated axon number as well as an increase in myelin thickness.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Myelin Formation Following Sciatic Nerve Cryolesion

15 Cryolesion of the mouse sciatic nerve was performed according to Koenig et al., *Science*, Vol. 268, pp. 1500-1503 (June 1995). In brief, each mouse was anesthetized and its sciatic nerve was exposed in the upper thigh and cryolesioned using a copper cryode (diameter = 0.5 mm)
20 that was dipped in liquid nitrogen and repeatedly applied to the upper part of the nerve. The extent of the lesion was approximately 1 mm.

 2-(Phosphonomethyl)pentanedioic acid was incorporated into silicone strips according to the method
25 of Connold et al., *Developmental Brain Res*, Vol. 28, pp. 99-104 (1986), and was implanted at the site of

cryolesion on day 0 and replaced on days 3, 6, 9 and 12. Approximately 2.5 μ g/day of 2-(phosphonomethyl)-pentanedioic acid was released from the silicone implants each day. Both right and left sciatic nerves of each mouse were lesioned; right-side nerves were treated with silicone implant strips containing vehicle alone while left-side nerves were treated with silicone implants containing 2-(phosphonomethyl)pentanedioic acid. Fifteen days after surgery, the mice were sacrificed and their sciatic nerve segments were collected and processed for light microscopy and TEM analysis. Randomly chosen fields 2-3 mm distal to the lesion were qualitatively analyzed by light microscopy using 1-micrometer-thick toluidine blue stained cross sections and photographic images were captured.

In Vivo Assay of NAALADase Inhibitors on Parkinson's Disease

To examine the effect of NAALADase inhibitors on Parkinson's Disease *in vivo*, MPTP lesioned mice were treated with 2-(phosphonomethyl)pentanedioic acid or a vehicle.

The percent of dopaminergic neurons for each group of mice is provided below in TABLE X and graphically presented in FIG. 11.

TABLE X
IN VIVO EFFECT OF NAALADASE INHIBITORS ON PARKINSON'S
DISEASE

5	Percent Strial TH Innervation Density (mean \pm SEM)
vehicle/vehicle	24.74 \pm 1.03
10	MPTP/vehicle
	7.82 \pm 0.68
	MPTP/2- (phosphonomethyl) - pentanedioic acid
15	16.28 \pm 0.98

Mice treated with MPTP and vehicle exhibited a substantial loss of functional dopaminergic terminals as compared to non-lesioned mice (approximately 68% loss). Lesioned mice receiving 2- (phosphonomethyl)pentanedioic acid (10 mg/kg) showed a significant recovery of TH-stained dopaminergic neurons ($p < 0.001$). These results indicate that 2- (phosphonomethyl)pentanedioic acid protects against MPTP-toxicity in mice.

25

Protocol for In Vivo Assay of NAALADase Inhibitors on
Parkinson's Disease

MPTP lesioning of dopaminergic neurons in mice was used as an animal model of Parkinson's Disease, as described by Steiner, *Proc. Natl. Acad. Sci.*, Vol. 94, pp. 2019-2024 (March 1997). In brief, four week old male CD1 white mice were dosed IP with 30 mg/kg of MPTP for 5 days. 2-(Phosphonomethyl)pentanedioic acid (10 mg/kg) or a vehicle was administered SC along with the MPTP for 5 days, as well as for an additional 5 days following cessation of MPTP treatment. At 18 days following MPTP treatment, the mice were sacrificed and their brains were removed and sectioned. Immunostaining was performed on sagittal and coronal brain sections using anti-tyrosine hydroxylase (TH) antibodies to quantitate survival and recovery of dopaminergic neurons.

In Vivo Assay of NAALADase Inhibitors on Dynorphin-
Induced Spinal Cord Injury

To examine the neuroprotective effect of NAALADase inhibitors on excitotoxic spinal cord injury *in vivo*, rats which had sustained dynorphin-induced spinal cord injury were treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid.

The results are graphically presented in FIG. 12. When co-administered with dynorphin A, 2-

(phosphonomethyl)pentanedioic acid (4 μ moles) caused significant improvement in motor scores by 24-hour post-injection, as compared to vehicle treated rats ($p < 0.05$, Kruskal-Wallis comparison). The rats were characterized as ambulatory or not on the basis of their assigned neurological scores (0 to 4). At 24 hours post-injection, 73% of the 15 rats co-treated with 2-(phosphonomethyl)pentanedioic acid were ambulatory, in contrast to 14% of the 14 vehicle co-treated rats ($p < 0.05$). These results indicate that 2-(phosphonomethyl)pentanedioic acid provides effective protection against dynorphin-induced spinal cord injury.

Protocol for In Vivo Assay of NAALADase Inhibitors on

Dynorphin-Induced Spinal Cord Injury

Spinal Subarachnoid Injections

Dynorphin-induced spinal cord injury was performed according to Long et al., *JPET*, Vol. 269, No. 1, pp. 358-366 (1993). In brief, spinal subarachnoid injections were delivered using 30-gauge needles inserted between the L4-L5 vertebrae of male Sprague-Dawley rats (300-350 g). The rats were anesthetized with halothane and dorsal midline incisions were made immediately rostral to the pelvic girdle. By using the vertebral processes as guides, the needle was advanced to pass into the subarachnoid space surrounding the cauda equina. Correct

needle placement was verified by CSF flow from the needle after its insertion. Injections were delivered using a Hamilton microsyringe in a total volume of 20 μ l which contained dynorphin (20 nmol), the cannula flush and 2-
5 (phosphonomethyl)pentanedioic acid or vehicle. After injections, the incisions were treated with the topical antibacterial furazolidone and closed with wound clips. Rapid recovery from the halothane anesthesia enabled neurological evaluations to be made within 5 minutes of
10 injections:

Neurological Evaluations

Neurological function was evaluated using a 5-point ordinal scale, with scores being assigned as follows: 4 = normal motor function; 3 = mild paraparesis, with the
15 ability to support weight and walk with impairment; 2 = paraparesis, with the ability to make walking movements without fully supporting weight; 1 = severe paraparesis, in which rats could make limited hind limb movement, but not walking movement; and 0 = flaccid paralysis, with
20 complete absence of any hind limb movement. Neurological evaluations were made 24 hours after dynorphin A injection.

Statistics

Differences in the neurological scores among
25 treatment groups were determined by means of the Mann-Whitney U test or the Kruskal-Wallis test.

In Vitro Assay of NAALADase Inhibitors on Amyotrophic
Lateral Sclerosis (ALS)

To examine the neuroprotective effect of NAALADase inhibitors on Amyotrophic Lateral Sclerosis (ALS), spinal cord organotypic cultures were treated with threohydroxyaspartate (THA), 2-(phosphonomethyl)-pentanedioic acid, or THA combined with 2-(phosphonomethyl)pentanedioic acid, and assayed for choline acetyltransferase (ChAT) activity.

The ChAT activity for each treatment of the spinal cord organotypic cultures is provided below in TABLE XI and graphically presented in FIG. 13.

TABLE XI
NEUROPROTECTIVE EFFECT OF NAALADASE INHIBITORS IN
SPINAL CORD CULTURE MODEL OF ALS

5	Treatment	ChAT Activity
		(% of Control)
	control	100 ± 22.1
10	2-(phosphonomethyl)- pentanedioic acid alone	108 ± 18.4
	THA alone	36 ± 12.1
15	2-(phosphonomethyl)- pentanedioic acid and THA	121 ± 18.8

20

As shown in FIG. 13, treatment of the spinal cord organotypic cultures with 100 μ M THA resulted in a reduction of ChAT activity to approximately 36% of control cultures. Co-incubation of the cultures with THA and 2-(phosphonomethyl)pentanedioic acid (100 nM - 10 μ M) significantly protected the cultures from THA toxicity.

25

The dose-response of this effect is provided below in TABLE XII and graphically presented in FIG. 14.

TABLE XII

NEUROPROTECTIVE EFFECT OF NAALADASE INHIBITORS IN
SPINAL CORD CULTURE MODEL OF ALS

	ChAT Activity (% of Control)
control	100.0
THA	0
THA and 1 nM 2-(phosphonomethyl)- pentanedioic acid	-23.9 ± 18.6
THA and 10 nM 2-(phosphonomethyl)- pentanedioic acid	23.1 ± 12.5
THA and 100 nM 2-(phosphonomethyl)- pentanedioic acid	87.5 ± 21.7
THA and 1 μM 2-(phosphonomethyl)- pentanedioic acid	187.7 ± 32.8

THA and 10 μ M 2-(phosphonomethyl)- 128.7 \pm 17.2
pentanedioic acid

5 Spinal cord cultures were incubated with various
doses of 2-(phosphonomethyl)pentanedioic acid (1 nM to 10
 μ M) in the presence of THA (100 μ M) for 14 days. As
shown in FIG. 14, 2-(phosphonomethyl)pentanedioic acid
exhibited dose-dependent protection against THA-induced
10 toxicity with maximal effects at 1 μ M.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Amyotrophic Lateral Sclerosis (ALS)

Spinal Cord Organotypic Cultures

15 Organotypic cultures were prepared from lumbar
spinal cord of 8 day old rats, as described by Rothstein
et al., *J. Neurochem.*, Vol. 65, No. 2 (1995), and
Rothstein et al., *Proc. Natl. Acad. Sci. USA*, Vol. 90,
pp. 6591-6595 (July 1993). In brief, lumbar spinal cords
20 were removed and sliced into 300 μ M-thick-dorsal-ventral
sections, and five slices were placed on Millipore CM
semipermeable 30-mm-diameter membrane inserts. The
inserts were placed on 1 ml of culture medium in 35-mm-
diameter culture wells. Culture medium consisted of 50%
25 minimal essential medium and phosphate-free HEPES (25
mM), 25% heat-inactivated horse serum, and 25% Hanks'

balanced salt solution (GIBCO) supplemented with D-glucose (25.6 mg/ml) and glutamine (2 mM), at a final pH of 7.2. Antibiotic and antifungal agents were not used. Cultures were incubated at 37° C in 5% CO₂ containing humidified environment (Forma Scientific). Culture medium, along with any added pharmacological agents, was changed twice weekly.

Chronic Toxicity Model with THA

For all experiments, cultures were used 8 days after preparation at which time threohydroxyaspartate (THA; 100 μ M), 2-(phosphonomethyl)pentanedioic acid (100 pM - 10 μ M), or THA (100 μ M) \pm 2-(phosphonomethyl)pentanedioic acid (100 pM - 10 μ M) were added to the culture medium. Drugs were incubated for an additional 13 to 20 days with the 100 μ M THA. At the end of this period, cultures were collected assayed for ChAT activity as described below.

ChAT Assays

To determine choline acetyltransferase (ChAT) activity, the spinal cord tissues in each dish (five slices) were pooled and frozen (-75° C) until assay. ChAT activity was measured radiometrically by described methods using [³H]acetyl-CoA (Amersham; Fonnum, 1975). Protein content of tissue homogenate was determined by a Coomassi Protein Assay kit (Pierce, Rockford, IL).

In Vivo Assay of NAALADase Inhibitors on EthanolConsumption in Alcohol-Preferring Rats

To test the effect of NAALADase inhibitors on ethanol consumption, alcohol-preferring rats were treated with saline or a 50, 100 or 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid prior to ethanol access. The ethanol intake of the rats following treatment is graphically presented in FIG. 15.

As shown in FIG. 15, the 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid exhibited no effect, whereas both the 50 and 100 mg/kg doses significantly reduced ethanol consumption by approximately 25% ($p < 0.05$) during the 1 hour access period. Body weights and 24 hour water intakes were not altered at any of the 3 doses. If 2-(phosphonomethyl)pentanedioic acid is acting centrally, these data suggest that NAALADase may be involved in neuronal systems regulating alcohol-drinking behavior.

Saline Baseline: 8.9 ± 0.7

200 mg/kg 2-(phosphonomethyl)pentanedioic acid:

8 ± 0.5

Saline Baseline: 7.8 ± 0.8

100 mg/kg 2-(phosphonomethyl)pentanedioic acid:

5.8 ± 0.7

Saline Baseline: 8.1 ± 0.6

50 mg/kg 2-(phosphonomethyl)pentanedioic acid:

6.2 ± 0.9

5 Protocol for In Vivo Assay of NAALADase Inhibitors on
 Ethanol Consumption in Alcohol-Preferring Rats

 The effect of systemic administration of 2-(phosphonomethyl)pentanedioic acid was examined on ethanol intake in the alcohol-preferring (P) line of rats, as described by Panocka et al., *Pharm. Biochem. and Behavior*, Vol. 52, No. 2, pp. 255-259 (1995) and Murphy et al., *Alcohol*, Vol. 2, pp. 349-352 (1985). In brief, 2-(phosphonomethyl)pentanedioic acid (50, 100 and 200 mg/kg IP) was tested in female P rats (n = 8) given daily 1 hour scheduled access to a 10% (v/v) ethanol solution. A within-subject design was used where 2-(phosphonomethyl)pentanedioic acid treatments were tested once per week. Baseline ethanol drinking consisted of the mean of the 3 days prior to testing in which saline injections were given. 2-(Phosphonomethyl)pentanedioic acid or saline, administered IP in 1 ml/kg volumes, were injected 10-15 minutes prior to ethanol access. 24 hour water and daily body weights were recorded to assess non-specific drug effects. Results were analyzed using paired t-tests with baseline and test day values serving as the independent variables. Ethanol intake was

recorded as amount of solution consumed (mls).

In Vivo Assay of NAALADase Inhibitors on Nicotine Self-
Administration in Male Long-Evans Rats

5 To test the effect of NAALADase inhibitors on
nicotine self-administration, male Long-Evans rats
trained to self-administer nicotine were treated with a
200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid
prior to nicotine access. The cumulative nicotine intake
10 of the rats following treatment is graphically presented
in FIG. 16.

 The results show that the 200 mg/kg dose of 2-
(phosphonomethyl)pentanedioic acid reduced nicotine self-
administration from 23 to 5 infusions during the 1 hour
15 access period. As graphically presented in FIG. 17, the
cumulative food intake of the rats also decreased during
the same period of time. While these data suggest that
factors other than 2-(phosphonomethyl)pentanedioic acid
may be responsible for the reduction in nicotine self-
20 administration, they do not disprove NAALADase's
involvement in the neuronal systems regulating nicotine
use. The effect on the rats' food intake could be
attributed to toxicity caused by an excessive drug dose.

25

Protocol for In Vivo Assay of NAALADase Inhibitors on
Nicotine Self-Administration in Male Long-Evans Rats

Male Long-Evans rats were trained to self-administer nicotine on a fixed ratio schedule of reinforcement, as described by Corrigall et al., *Psychopharmacology*, Vol. 104, No. 2, pp. 171-176 (1991) and Corrigall et al., *Psychopharmacology*, Vol. 107, Nos. 2-3, pp. 285-289 (1992). In brief, male Long-Evans rats were food deprived for a short period of time (24-48 hours) and trained to press a lever in an operant responding chamber on an FR-1 schedule of food reinforcement. Once trained, each rat was surgically prepared with a chronic intravenous catheter implanted into the jugular vein. The rats were allowed 1 week to recover from surgery.

After 1 week, nicotine self-administration studies were initiated on an FR-1 with a 60 second signaled time-out following each infusion. During time-out, responding on the lever had no scheduled consequence. Nicotine self-administration sessions were 60 minutes in duration. Each nicotine infusion contained 30 μ g of nicotine/kg rat and were delivered in a volume of 54 μ l over an infusion duration of 0.3 seconds. 15 minutes before the self-administration sessions, the rats were pre-treated intraperitoneally with 2-(phosphonomethyl)-pentanedioic acid at doses of 10, 20 and 30 mg/kg. Food intake was monitored during the nicotine self-administration

sessions to assess non-specific drug effects.

In Vitro Assay of NAALADase Inhibitors
on Cancer

5 To examine the effect of NAALADase inhibitors on
cancer cell line, LNCAP cells (a prostate cancer cell
line) were treated with quisqualate acid (in
concentrations ranging from 10 nM to 1 μ M) and 2-
(phosphonomethyl)pentanedioic acid (in concentrations
10 ranging from 100 pM to 10 nM). The ³H-thymidine
measurement for each concentration of quisqualate acid
and 2-(phosphonomethyl)pentanedioic acid is provided in
TABLE XIII below and graphically represented in FIG. 18
and FIG. 19, respectively.

15

TABLE XIII

<u>3H-Thymidine Incorporation (dpm/well)</u>			
<u>Dose</u>	<u>Quisqualic Acid</u>	<u>2-(phosphonomethyl)- pentanedioic acid</u>	
20 Control	4813 \pm 572	4299 \pm 887	
10 pM	--	3078 \pm 1006	
25 100 pM	--	2062 \pm 595	
1 nM	3668 \pm 866	1001 \pm 52	
10 nM	2137 \pm 764	664 \pm 366	
30 100 nM	1543 \pm 312	--	
1 μ M	1295 \pm 181	--	

The results show that LNCAP cell proliferation (as measured by the incorporation of 3H-thymidine) decreased significantly as the concentration of the NAALADase inhibitors increased, suggesting that the compounds of the present invention would be effective in treating cancer, particularly prostate cancer.

Protocol for In Vitro Cancer Assay

Cells in RPMI 1640 medium containing 10% Fetal Calf Serum (FCS) are plated in 24 well plates and allowed to adhere for 24 hours before addition of quisqualic acid (10^{-9} to 10^{-6}) or 2-(phosphonomethyl)pentanedioic acid (10^{-11} to 10^{-8}) for 7 days. On the 7th day, the cells are pulsed with 3H-thymidine for 4 hours, harvested and measured for radioactivity. Values represent means \pm SEM of 6 separate cell wells for each treatment. All experiments are performed at least twice.

To control for non-specific cytostatic effects of quisqualate acid and 2-(phosphonomethyl)pentanedioic acid, the agents are simultaneously evaluated on a non-NAALADase containing prostate cell line, DU145 (Carter et al., Proc. Natl. Acad. Sci. USA, (93) 749-753, 1996). If the treatments with quisqualate acid and 2-(phosphonomethyl)pentanedioic have no significant effect on cell growth, the NAALADase inhibiting activity of the agents are uniquely responsible for their cytostatic

effects on prostate carcinoma cell lines.

Cell Lines and Tissue Culture

LNCAP cells are obtained from Dr. William Nelson at the Johns Hopkins School of Medicine in Baltimore, MD. DU145 cells are obtained from American Type Culture Collection (Rockville, MD). Cells are grown in RPMI-1640 media supplemented with 5% heat-inactivated fetal calf serum, 2 mM-glutamine, 100 units/ml penicillin, and 100 μ g/ml streptomycin (Paragon) in a humidified incubator at 37°C in a 5% CO₂/95% air atmosphere.

[3H] Thymidine Incorporation Assays

The cells are suspended at 1×10^3 cells/ml in RPMI-1640 media and seeded into 24-well plates at 500 μ l per well. After 24 hour incubation, various concentrations of quisqualic acid (Sigma) or the potent NAALADase inhibitor 2-(phosphonomethyl)pentanedioic acid (synthesized according to the methods of Jackson et al., *J. Med. Chem.*, Vol. 39, No. 2, pp. 619-622, is added to the wells and the plates are returned to the incubator. On days 3, 5 and 7, media and drug are refreshed. On the 8th day following seeding, each well is pulsed with 1 μ Ci ³H-thymidine (New England Nuclear) for 4 hours. Media is then removed and the wells washed 2 times with phosphate buffered saline (pH=7.4). The contents of each well is subsequently solubilized 250 μ l of 0.2 N NaOH and transferred to scintillation vials. 5 ml UltimaGold

(Packard) scintillation cocktail is added and radioactivity is quantitated using a Beckman LS6001 scintillation counter.

5 The purity and/or identity of all synthetic compounds is ascertained by thin layer chromatography, High Pressure Liquid Chromatography (HPLC), mass spectrometry, and elemental analysis. Proton Nuclear Magnetic Resonance (NMR) spectra are obtained using a Bruker spectrometer. Chemical shifts are reported in
10 parts per million relative to tetramethylsilane as internal standard. Analytical thin-layer chromatography (TLC) is conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE). Visualization of the plates is accomplished by using UV light, phosphomolybdic acid-
15 ethanol, and/or iodoplatinate charring. Flash chromatography is conducted on Kieselgel 60, 230-400 mesh (E. Merck, Darmstadt, West Germany). Solvents are either reagent or HPLC grade. Reactions are run at ambient temperature and under a nitrogen atmosphere unless
20 otherwise noted. Solutions are evaporated under reduced pressure on a Buchi rotary evaporator.

EXAMPLES

25 The following examples are illustrative of the present invention and are not intended to be limitations thereon. Unless otherwise indicated, all percentages are

based upon 100% by weight of the final composition.

EXAMPLE 1

Synthesis of 2-[[[2-(carboxy)propyl]hydroxy- phosphinyl]methyl]pentanedioic acid

Di-tert-butyl 2-methylenepentanedioate

Tert-butyl acrylate (465 g, 3.628 mol) was warmed to 100° C under nitrogen, then hexamethylphosphorous triamide (10 g, 61.2 mmol) was added dropwise and the addition rate was adjusted to maintain the reaction temperature at 100° C. The reaction mixture was allowed to cool, then poured over a plug of silica (~1000 ml) and washed completely off the silica with 4:1 hexane/ethyl acetate. The solvent was removed under reduced pressure and the resulting oil was distilled. Some material was collected from room temperature to 50° C under high vacuum, and discarded. The temperature was then raised to ~80° C and the product (300 g, 65 %, b.p. 67-70° C at 300μ) was collected as a clear oil. ¹H NMR (CDCl₃): δ 1.4 (m, 18H), 2.4 (t, 2H), 2.6 (t, 2H), 5.5 (s, 1H), 6.0 (s, 1H).

Di-tert-butyl 2-[(hydroxyphosphinyl)methyl]pentanedioate

A mixture of ammonium phosphinate (162.6 g, 1.96 mol) and 1,1,1,3,3,3-hexamethyldisilazane (316 g, 1.96 mol) was heated to 105° C for 2 hours. The reaction mixture was cooled in an ice bath and di-tert-butyl 2-

methylenepentane-1,5-dioate (251 g, 0.979 mol) dissolved in dichloromethane (1000 ml) was added dropwise. The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was then quenched with
5 distilled water (500 ml) and the organic layer was retained. The aqueous layer was washed a second time with dichloromethane and the combined organic layers were dried over magnesium sulfate. Then the solvent was removed under reduced pressure leaving a slightly yellow
10 oil (315 g, 100 %). This product was found to be of sufficient purity for use in the next reaction. ¹H NMR (CDCl₃): δ 1.4 (m, 18H), 1.9 (m, 3H), 2.1 (m, 1H), 2.3 (m, 2H), 2.7 (m, 1H), 6.5 & 7.9 (d, 1H, the P-H), 11.0 (s, 1H).

15 Di-tert-butyl 2-[(tert-butoxyphosphinyl)methyl]-pentanedioate

To a solution of di-tert-butyl 2-[(hydroxyphosphinyl)methyl]pentane-1,5-dioate (315 g, 0.977 mol) in dichloromethane (1000 ml) cooled in an ice bath and
20 under nitrogen were added tert-butanol (123.1 g, 1.66 mol), 4-dimethylaminopyridine (1 g, 8.2 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (281 g, 1.47 mol). The reaction was allowed to stir overnight. Water was added to the reaction mixture and the dichloromethane
25 layer was retained and dried, and the solvent was removed under reduced pressure. The resulting residue was

purified by column chromatography and the desired product was eluted with 1:1 to 2:3 hexane/ethyl acetate. The fractions containing product were concentrated under reduced pressure leaving a clear oil (260 g, 70 %). ¹H NMR (CDCl₃): δ 1.4 (m, 27H), 1.8 (m, 1H), 1.9 (m, 2H), 2.1 (m, 1H), 2.3 (m, 2H), 2.7-2.8 (m, 1H), 6.7 & 8.0 (d, 1H, the P-H).

Di-tert-butyl 2-[[[2-(benzylcarboxy)propyl]tert-butoxyphosphinyl]methyl]pentanedioate

To a solution of di-tert-butyl 2-[(tert-butoxyphosphinyl)methyl]pentane-1,5-dioate (13.62 g, 36.0 mmol) and benzyl methacrylate (6.35 g, 36.0 mmol) in THF (100 ml) under nitrogen was added sodium hydride (0.14 g, 60 % dispersion in oil, 3.60 mmol). After three hours, the reaction mixture was poured into water (300 ml) and ether (100 ml) was added. The organic layer was separated and retained, and the aqueous layer was washed again with ether (100 ml). The combined organic extracts were dried over magnesium sulfate and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography and the product was eluted with 2:3 EtOAc/Hexane. The solvent was removed under reduced pressure leaving a clear oil (10.5 g, 53 %). ¹H NMR (CDCl₃): δ 1.3 (m, 3H), 1.5 (m, 27H), 1.7 (m, 2H), 1.9 (m, 2H), 2.2 (m, 4H), 2.6 (m, 1H), 2.9 (m, 1H), 5.1 (m, 2H), 7.3 (m, 5H).

2-[[[2-(Benzylcarboxy)propyl]hydroxyphosphinyl]-methyl]pentanedioic acid

To a solution of di-tert-butyl 2-[[[2-(benzylcarboxy)propyl]tert-butoxyphosphinyl]methyl]pentane-1,5-dioate (1.6 g, 2.89 mmol) in dichloromethane (10 ml) under nitrogen was added trifluoroacetic acid (10 ml). The reaction mixture was stirred for two hours and then concentrated under reduced pressure. Additional dichloromethane was added to the reaction residue and removed under reduced pressure. The product was dissolved in ethyl acetate and washed with water, then the organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure leaving a clear oil (800 mg, 72 %). ¹H NMR (D₂O): δ 1.2 (m, 3H), 1.6-1.8 (m, 4H), 2.1 (m, 2H), 2.2 (m, 2H), 2.6 (m, 1H), 2.8 (m, 1H), 5.0 (m, 2H), 7.3 (m, 5H). Analysis calculated for C₁₇H₂₃PO₈ · 1.0 H₂O: C, 50.50; H, 6.23. Found: C, 50.52; H, 5.92.

Di-tert-butyl 2-[[[2-(carboxy)propyl]tert-butoxyphosphinyl]methyl]pentanedioate

A solution of di-tert-butyl 2-[[[2-(benzylcarboxy)propyl]tert-butoxyphosphinyl]methyl]pentane-1,5-dioate (8.9 g, 16.1 mmol), palladium on carbon catalyst (10 %, 1.0 g) and ethyl acetate (100 ml) was shaken under hydrogen (60 psi) for 16 hours. The reaction mixture was filtered over celite and the filtrate was concentrated

under reduced pressure leaving a clear oil (7.5 g, 100 %). ^1H NMR (CDCl_3): δ 1.3 (d, 3H), 1.4-1.5 (m, 27H), 1.8 (m, 2H), 1.9 (m, 2H), 2.2 (m, 4H), 2.7 (m, 1H), 2.9 (m, 1H).

5 2-[[[2-(Carboxy)propyl]hydroxyphosphinyl]methyl]-pentanedioic acid

To a solution of di-tert-butyl 2-[[[2-(carboxy)-propyl]tert-butoxyphosphinyl]methyl]pentane-1,5-dioate (2.1 g, 4.53 mmol) in dichloromethane (10 ml) under
10 nitrogen was added trifluoroacetic acid (10 ml). The reaction mixture was stirred for two hours and then concentrated under reduced pressure. Additional dichloromethane was added to the reaction residue and removed under reduced pressure. The resulting residue
15 was triturated with acetonitrile, then dried under reduced pressure leaving a thick clear oil (1.2 g, 89 %). ^1H NMR (D_2O): δ 1.2 (d, 3H), 1.9 (m, 4H), 2.2 (m, 2H), 2.4 (m, 2H), 2.8 (m, 2H). Analysis calculated for $\text{C}_{10}\text{H}_{17}\text{PO}_8 \cdot 0.2 \text{ CH}_3\text{CN}$: C, 41.03; H, 5.83. Found: C, 41.05;
20 H, 5.92.

EXAMPLE 2

A patient is at risk of injury from an ischemic event. The patient may be pretreated with an effective
25 amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the

pretreatment, the patient would be protected from any injury due to the ischemic event.

EXAMPLE 3

5 A patient is suffering from an ischemic event. The patient may be administered during or after the event, an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would recover or
10 would not suffer any significant injury due to the ischemic event.

EXAMPLE 4

15 A patient has suffered injury from an ischemic event. The patient may be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would recover from the injury due to the ischemic event.

20

EXAMPLE 5

 A patient is suffering from a glutamate abnormality. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the
25 present invention. It is expected that after the treatment, the patient would be protected from further

injury due to the glutamate abnormality or would recover from the glutamate abnormality.

EXAMPLE 6

5 A patient is suffering from or has suffered from a nervous insult, such as that arising from a neurodegenerative disease or a neurodegenerative process. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further injury due to the nervous insult or would recover from the nervous insult.

EXAMPLE 7

15 A patient is suffering from Parkinson's disease. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from Parkinson's disease.

EXAMPLE 8

25 A patient is suffering from ALS. The patient may then be administered an effective amount of a compound or

a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from ALS.

5

EXAMPLE 9

A patient is suffering from epilepsy. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from epilepsy.

EXAMPLE 10

A patient is suffering from abnormalities in myelination/demyelination processes. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from the abnormalities in myelination/demyelination processes.

EXAMPLE 11

A patient is suffering from or has suffered from a cerebrovascular accident, such as stroke. The patient

may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any injury due to the cerebrovascular accident.

EXAMPLE 12

A patient is suffering from a head trauma. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury resulting from the head trauma.

EXAMPLE 13

A patient is suffering from a spinal trauma. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic injury resulting from the spinal trauma.

EXAMPLE 14

A patient is about to undergo surgery. The patient may be administered an effective amount of a compound or

a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would not develop any ischemic brain, spinal or peripheral injury resulting from or associated with the surgery.

EXAMPLE 15

A patient is suffering from focal ischemia, such as that associated with thromboembolytic occlusion of a cerebral vessel, traumatic head injury, edema or brain tumors. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any brain, spinal or peripheral injury resulting from the focal ischemia.

EXAMPLE 16

A patient is suffering from global ischemia. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any brain, spinal or peripheral injury resulting from the global ischemia.

EXAMPLE 17

5 A patient is suffering from a cardiac arrest. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury associated with the cardiac arrest.

10

EXAMPLE 18

15 A patient is suffering from hypoxia, asphyxia or perinatal asphyxia. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury associated with the hypoxia, asphyxia or perinatal asphyxia.

20

EXAMPLE 19

25 A patient is suffering from a cerebro-cortical injury. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain injury

resulting from the cerebro-cortical injury.

EXAMPLE 20

5 The patient is suffering from an injury to the caudate nucleus. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain injury
10 resulting from the injury to the caudate nucleus.

EXAMPLE 21

A patient is suffering from a cortical injury due to a condition identified in these examples. The patient
15 may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further injury, or would exhibit at least 65% to at least 80% recovery from the
20 cortical injury.

EXAMPLE 22

A patient is suffering from multiple sclerosis. The patient may then be administered an effective amount of
25 a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the

patient would be protected from further demyelination or would recover from multiple sclerosis.

EXAMPLE 23

5 A patient is suffering from a peripheral neuropathy caused by Guillain-Barré syndrome. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would
10 be protected from further demyelination or would recover from the peripheral neuropathy.

EXAMPLE 24

 The patient is suffering from alcoholism. The
15 patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for alcohol would be suppressed.

EXAMPLE 25

20 A patient is suffering from nicotine dependence. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the
25 treatment, the patient's craving for nicotine would be suppressed.

EXAMPLE 26

The patient is suffering from cocaine dependence. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for cocaine would be suppressed.

EXAMPLE 27

A patient is suffering from heroine dependence. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for heroine would be suppressed.

EXAMPLE 28

The patient is suffering from compulsive overeating, obesity or severe obesity. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's compulsion to eat would be suppressed.

EXAMPLE 29

A patient is suffering from pathological gambling. The patient may then be administered an effective amount

of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's compulsion to gamble would be suppressed.

5

EXAMPLE 30

The patient is suffering from ADD. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's symptoms of inattention, impulsivity and/or hyperactivity would be suppressed.

10

EXAMPLE 31

A patient is suffering from Tourette's syndrome. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's simple, complex, respiratory and vocal tics would be suppressed.

15

20

EXAMPLE 32

A patient is suffering from adenocarcinoma of the prostate. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention. After this initial

25

treatment, the patient may optionally be administered the same or a different compound of the present invention in intermittent or continuous doses by subdural pump. It is expected that the treatment(s) would prevent recurrences of the adenocarcinoma, or inhibit (i.e., arrest development of) or relieve (i.e., cause regression of) the adenocarcinoma tumor cells.

EXAMPLE 33

A patient is suffering from adenocarcinoma of the prostate. The patient may then be administered an effective amount of a compound or a pharmaceutical composition of the present invention by direct injection into the tumor. After this initial treatment, the patient may optionally be administered an effective amount of the same or a different compound of the present invention in intermittent or continuous doses by implantation of a biocompatible polymeric matrix delivery system. It is expected that the treatment(s) would prevent recurrences of the adenocarcinoma, or inhibit (i.e., arrest development of) or relieve (i.e., cause regression of) the adenocarcinoma tumor cells.

EXAMPLE 34

A patient is diagnosed with benign prostatic hyperplasia. The patient may then be administered an

effective amount of a compound or a pharmaceutical composition of the present invention by direct injection into the tumor. After this initial treatment, the patient may optionally be administered the same or a different compound of the present invention in intermittent or continuous doses by injection, subdural pump or polymeric matrix implant. It is expected that after the treatment(s), the benign prostatic hyperplastic cells would not develop into carcinoma.

10

EXAMPLE 35

A patient is suffering from adenocarcinoma of the prostate. The adenocarcinoma does not appear to have metastasized. The patient undergoes surgery to remove the adenocarcinoma. After post-surgical recovery, the patient may be locally administered an effective amount of a compound or a pharmaceutical composition of the present invention in intermittent or continuous doses by injection, subdural pump or polymeric matrix implant. It is expected that after the treatment, the patient would be protected from recurrences of the adenocarcinoma, and any residual tumorous cells would be inhibited (i.e., arrested in development) or relieved (i.e., caused to regress).

25

EXAMPLE 36

A patient is suffering from metastatic adenocarcinoma of the prostate. Although the adenocarcinoma appears to have metastasized, the patient nevertheless undergoes surgery to remove the adenocarcinoma. The patient may then be locally administered an effective amount of a compound or a pharmaceutical composition of the present invention approximately from the time of initial diagnosis through post-surgical recovery. After post-surgical recovery, the patient may continue the same treatment by a regimen of periodic local administration, and carefully monitored for adverse side-effects. It is expected that after the treatments, the patient would be protected from recurrences of the adenocarcinoma, and any residual tumorous cells would be inhibited (i.e., arrested in development) or relieved (i.e., caused to regress).

EXAMPLE 37

A patient is suffering from cancer as defined herein. An effective amount of a compound or a pharmaceutical composition of the present invention may be administered directly to the cancer cells. After this initial treatment, the patient may be optionally administered an effective amount of the same or a different compound of the present invention by direct

injection, subdural pump or implantation of a biocompatible polymeric matrix delivery system. It is expected that after the treatment(s), the patient would be protected from recurrences of the cancer, and the cancer would be inhibited (i.e., arrested in development) or relieved (i.e., caused to regress).

EXAMPLE 38

A patient is diagnosed with a disease, disorder or condition as identified in these examples. An effective amount of a compound or a pharmaceutical composition of the present invention may then be administered to the patient intravenously, intramuscularly, intraventricularly to the brain, rectally, subcutaneously, intranasally, through a catheter with or without a pump, orally, through a transdermal patch, topically, or through a polymer implant. After the treatment, the patient's condition would be expected to improve.

EXAMPLE 39

A patient is diagnosed with a disease, disorder or condition as identified in these examples. A compound or a pharmaceutical composition of the present invention may then be administered to the patient in the form of a 100 mg/kg bolus, optionally followed by a 20 mg/kg per hour

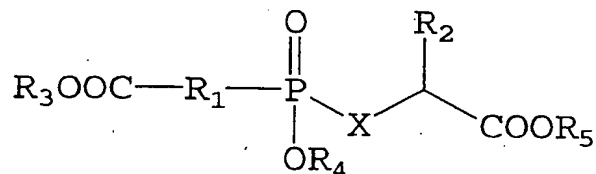
intravenous infusion over a two-hour period. After the treatment, the patient's condition would be expected to improve.

5 The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the
10 scope of the following claims.

WE CLAIM:

1. A compound of formula I:

5



I

10

or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

15

R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

20

25

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇

cycloalkenyl and Ar, wherein said R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_9 alkoxy, C_2 - C_9 alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuran-2-yl, tetrahydropyran-2-yl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, and amino.

20

2. The compound of claim 1, wherein X is CH_2 .

3. The compound of claim 2, wherein:

R_2 is $-(CH_2)_2COOR_9$; and

R_9 is selected from the group consisting of hydrogen, C_1 - C_9 straight or branched chain alkyl, C_2 - C_9

straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of
5 carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

10

4. The compound of claim 3, wherein R₃, R₄, R₅, and R₉ are hydrogen.

5. The compound of claim 3, which is selected from
15 the group consisting of:

2-[[2-carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic acid;

20 2-[[2-carboxypentyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

25 2-[[2-carboxy-3-naphthylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]-

pentanedioic acid;

2-[[2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]-
5 methyl]pentanedioic acid;

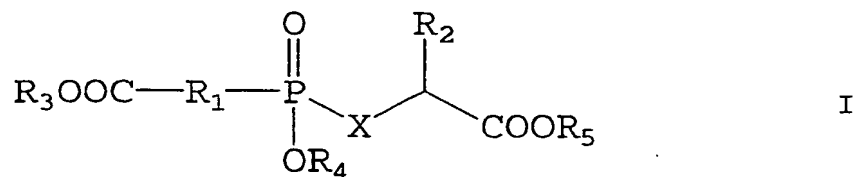
2-[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxyphosphinyl]methyl]pentanedioic acid; and

10 pharmaceutically acceptable salts, hydrates and prodrugs thereof.

6. The compound of claim 5, which is 2-[[2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid,
15 or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

7. A method of treating a glutamate abnormality in an animal, comprising administering to said animal an
20 effective amount of a compound of formula I:



25 or a pharmaceutically acceptable salt, hydrate or prodrug

thereof, wherein:

X is CR_6R_7 , O or NR_8 ;

R_1 is selected from the group consisting of $\text{C}_1\text{-C}_9$ straight or branched chain alkyl, $\text{C}_2\text{-C}_9$ straight or branched chain alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl and Ar, wherein said R_1 is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, $\text{C}_1\text{-C}_6$ straight or branched chain alkyl, $\text{C}_2\text{-C}_6$ straight or branched chain alkenyl, $\text{C}_1\text{-C}_9$ alkoxy, $\text{C}_2\text{-C}_9$ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently selected from the group consisting of hydrogen, $\text{C}_1\text{-C}_9$ straight or branched chain alkyl, $\text{C}_2\text{-C}_9$ straight or branched chain alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl and Ar, wherein said R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_5\text{-C}_7$ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, $\text{C}_1\text{-C}_6$ straight or branched chain alkyl, $\text{C}_2\text{-C}_6$ straight or branched chain alkenyl, $\text{C}_1\text{-C}_9$ alkoxy, $\text{C}_2\text{-C}_9$ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-

naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or
5 substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy,
10 benzyloxy, and amino.

8. The method of claim 7, wherein the compound is administered in combination with at least one additional therapeutic agent.

15

9. The method of claim 7, wherein the glutamate abnormality is selected from the group consisting of epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS),
20 Huntington's Disease, schizophrenia, chronic pain, ischemia, peripheral neuropathy, traumatic brain injury and physical damage to the spinal cord.

10. The method of claim 9, wherein the glutamate
25 abnormality is ischemia.

11. The method of claim 9, wherein the glutamate abnormality is stroke.

12. The method of claim 9, wherein the glutamate
5 abnormality is Parkinson's Disease.

13. The method of claim 9, wherein the glutamate abnormality is Amyotrophic Lateral Sclerosis (ALS).

10 14. The method of claim 9, wherein the glutamate abnormality is ischemic spinal cord injury.

15. The method of claim 7, wherein X is CH₂.

15 16. The method of claim 15, wherein:

R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted
20 or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or
25 branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy,

benzyloxy, amino, and Ar.

17. The method of claim 16, wherein R_3 , R_4 , R_5 , and R_9 are hydrogen.

5

18. The method of claim 16, wherein the compound is selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

10 2-[[[(2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl)methyl]pentanedioic acid;

15 2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

20 2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

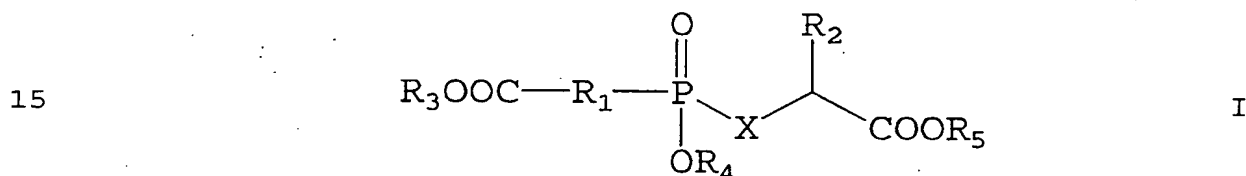
25 2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-

phosphinyl)methyl]pentanedioic acid; and
pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

5 19. The method of claim 18, wherein the compound is 2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid, or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

10 20. A method of effecting a neuronal activity in an animal, comprising administering to said animal an effective amount of a compound of formula I:



or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

20 X is CR_6R_7 , O or NR_8 ;

R₁ is selected from the group consisting of C₁-C₉, straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl,

C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain

alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

21. The method of claim 20, wherein the compound is administered in combination with at least one additional therapeutic agent.

22. The method of claim 20, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration, and treatment of a neurological disorder.

23. The method of claim 22, wherein the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke associated with brain damage, demyelinating disease and neurological disorder relating to neurodegeneration.

24. The method of claim 23, wherein the peripheral neuropathy is caused by Guillain-Barré syndrome.

25. The method of claim 23, wherein the demyelinating disease is multiple sclerosis.

26. The method of claim 23, wherein the neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, and amyotrophic lateral sclerosis.

27. The method of claim 20, wherein X is CH₂.

28. The method of claim 27, wherein:

10 R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

29. The method of claim 28, wherein R₃, R₄, R₅, and R₉ are hydrogen.

30. The method of claim 28, wherein the compound is

selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentane-
dioic acid;

2-[[[(2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic
5 acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl)methyl]pentane-
dioic acid;

2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]-
pentanedioic acid;

10 2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]-
methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]-
pentanedioic acid;

2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxy-
15 phosphinyl)methyl]pentanedioic acid;

2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;

2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;

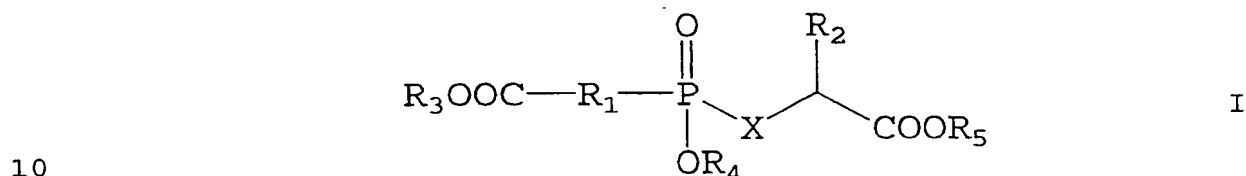
20 2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-
phosphinyl)methyl]pentanedioic acid; and

pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

25 31. The method of claim 30, wherein the compound is
2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentane-

dioic acid, or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

32. A method of treating a compulsive disorder,
 5 comprising administering to a patient in need thereof an effective amount of a compound of formula I:



or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

15 R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently
 20 selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and
 25 Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently

selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

33. The method of claim 32, wherein the compound is administered in combination with at least one additional therapeutic agent.

5

34. The method of claim 32, wherein the compulsive disorder is selected from the group consisting of drug dependence, eating disorders, pathological gambling, attention deficit disorder (ADD) and Tourette's syndrome.

35. The method of claim 34, wherein the drug dependence is alcohol dependence.

10

36. The method of claim 34, wherein the drug dependence is nicotine dependence.

37. The method of claim 30, wherein X is CH₂.

15

38. The method of claim 37, wherein:

R₂ is -(CH₂)₂COOR₉; and

20

R₉ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

25

39. The method of claim 38, wherein R_3 , R_4 , R_5 , and R_9 are hydrogen.

40. The method of claim 38, wherein the compound is
5 selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxybutyl)hydroxyphosphinyl]methyl]pentanedioic acid;

10 2-[[[(2-carboxypentyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

15 2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

20 2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl]methyl]pentanedioic acid;

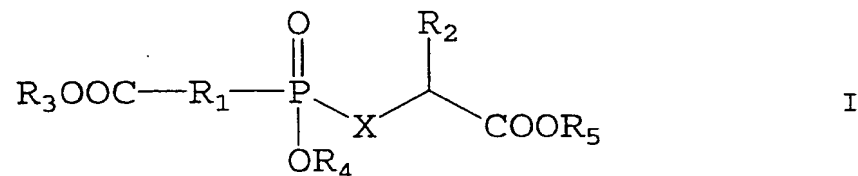
25 2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxyphosphinyl]methyl]pentanedioic acid; and

pharmaceutically acceptable salts, hydrates and prodrugs

thereof.

41. The method of claim 40, wherein the compound is
2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-
5 dioic acid, or a pharmaceutically acceptable salt,
hydrate or prodrug thereof.

42. A method of treating a prostate disease in an
animal, comprising administering to said animal an
10 effective amount of a compound of formula I:



15

or a pharmaceutically acceptable salt, hydrate or prodrug
thereof, wherein:

X is CR₆R₇, O or NR₈;

R₁ is selected from the group consisting of C₁-C₉,
20 straight or branched chain alkyl, C₂-C₉ straight or
branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl and Ar, wherein said R₁ is unsubstituted or
substituted with one or more substituent(s) independently
selected from the group consisting of carboxy, carbonyl,
25 C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy,
nitro, trifluoromethyl, C₁-C₆ straight or branched chain

alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar;

5 R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted
10 with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-
15 C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl,
20 benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain
25 alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

43. The method of claim 42, wherein the compound is administered in combination with at least one additional therapeutic agent.

5 44. The method of claim 42, wherein said prostate disease is prostate cancer or benign prostatic hyperplasia.

45. The method of claim 42, wherein X is CH₂.

10 46. The method of claim 43, wherein:

R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

25 47. The method of claim 46, wherein R₃, R₄, R₅, and R₉ are hydrogen.

48. The method of claim 46, wherein the compound is selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

5 2-[[[(2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl)methyl]pentanedioic acid;

10 2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

15 2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

20 2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxyphosphinyl)methyl]pentanedioic acid; and

pharmaceutically acceptable salts, hydrates and prodrugs thereof.

25

49. The method of claim 48, wherein the compound is

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

51. The method of claim 50, wherein the compound is administered in combination with at least one additional

therapeutic agent.

52. The method of claim 50, wherein X is CH₂.

5 53. The method of claim 52, wherein:

R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of
hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉
straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-
10 C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted
or substituted with one or more substituent(s)
independently selected from the group consisting of
carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl,
halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or
15 branched chain alkyl, C₂-C₆ straight or branched chain
alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy,
benzyloxy, amino, and Ar.

54. The method of claim 53, wherein R₃, R₄, R₅, and
20 R₉ are hydrogen.

55. The method of claim 53, wherein the compound is
selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentane-
25 dioic acid;

2-[[[(2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic

acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl]methyl]pentanedioic acid;

5 2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

10 2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic acid;

15 2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxyphosphinyl]methyl]pentanedioic acid; and

pharmaceutically acceptable salts, hydrates and prodrugs thereof.

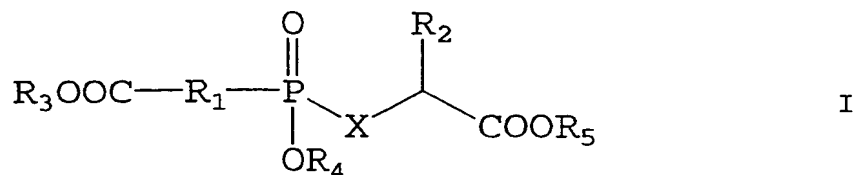
20

56. The method of claim 55, wherein the compound is 2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentanedioic acid, or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

25

57. A pharmaceutical composition comprising:

(i) an effective amount of a compound of formula I:



5

or a pharmaceutically acceptable salt, hydrate or prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

10 R₁ is selected from the group consisting of C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with one or more substituent(s) independently
 15 selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and
 20 Ar;

R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇
 25 cycloalkenyl and Ar, wherein said R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are independently unsubstituted or substituted

with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, and amino; and

(ii) a pharmaceutically acceptable carrier.

58. The pharmaceutical composition of claim 57, wherein X is CH₂.

59. The pharmaceutical composition of claim 58, wherein:

R₂ is -(CH₂)₂COOR₉; and

R₉ is selected from the group consisting of

hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₉ is unsubstituted or substituted with one or more substituent(s) independently selected from the group consisting of carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, and Ar.

60. The pharmaceutical composition of claim 59, wherein R₃, R₄, R₅, and R₉ are hydrogen.

61. The pharmaceutical composition of claim 59, wherein the compound is selected from the group consisting of:

2-[[[(2-carboxypropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxybutyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxypentyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-phenylpropyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(2-carboxy-3-naphthylpropyl)hydroxyphosphinyl]-

methyllpentanedioic acid;
2-[[[(2-carboxy-3-pyridylpropyl)hydroxyphosphinyl)methyl]-
pentanedioic acid;
2-[[[(2-benzyloxycarbonyl)-3-phenylpropyl)hydroxy-
5 phosphinyl)methyl]pentanedioic acid;
2-[[[(2-methoxycarbonyl)-3-phenylpropyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;
2-[[[(3-carboxy-2-methoxycarbonyl)propyl)hydroxy-
phosphinyl)methyl]pentanedioic acid;
10 2-[[[(4-carboxy-2-methoxycarbonyl)butyl)hydroxy-
phosphinyl)methyl]pentanedioic acid; and
pharmaceutically acceptable salts, hydrates and prodrugs
thereof.

15 62. The pharmaceutical composition of claim 61,
wherein the compound is 2-[[[(2-carboxypropyl)hydroxy-
phosphinyl)methyl]pentanedioic acid, or a
pharmaceutically acceptable salt, hydrate or prodrug
thereof.

20

63. The pharmaceutical composition of claim 57,
wherein the amount of the compound of formula I is
effective for inhibiting NAALADase enzyme activity in an
animal.

25

64. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for treating a glutamate abnormality in an animal.

5

65. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for effecting a neuronal activity in an animal.

10

66. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for treating a compulsive disorder in an animal.

15

67. The pharmaceutical composition of claim 57, wherein the amount of the compound of formula I is effective for treating a prostate disease in an animal.

AMENDED CLAIMS

[received by the International Bureau on 15 June 1999 (15.06.99);
original claims 1 and 50 amended; remaining claims unchanged (2 pages)]

cycloalkenyl and Ar, wherein said R_2 , R_3 , R_4 , R_5 , R_6 , R_7 ,
and R_8 are independently unsubstituted or substituted
with one or more substituent(s) independently selected
from the group consisting of carboxy, carbonyl, C_3 - C_6
5 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro,
trifluoromethyl, C_1 - C_6 straight or branched chain
alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6
alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, amino,
and Ar; provided that when X is $-\text{CH}_2-$, R_1 is $-(\text{CH}_2)_2-$,
10 R_2 is $-(\text{CH}_2)_2\text{CO}_2\text{CH}_2\text{C}_6\text{H}_5$, R_3 is benzyl, and R_4 is hydrogen,
then R_5 is not benzyl; and when X is $-\text{CH}_2-$, R_1 is $-(\text{CH}_2)_2-$,
 R_2 is $-(\text{CH}_2)_2\text{CO}_2\text{H}$, R_3 is hydrogen, and R_4 is
hydrogen, then R_5 is not hydrogen; and

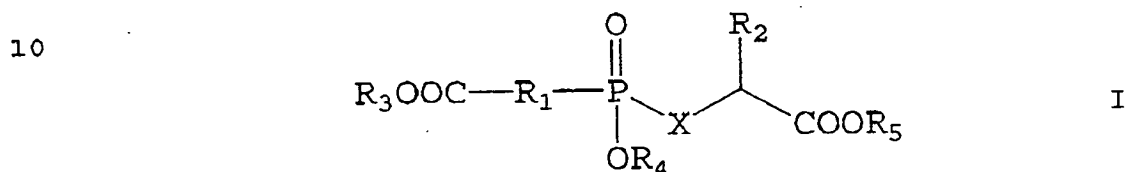
Ar is selected from the group consisting of 1-
15 naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl,
2-furyl, 3-furyl, tetrahydrofuranyl,
tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-
pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar
is unsubstituted or substituted with one or more
20 substituent(s) independently selected from the group
consisting of carboxy, carbonyl, halo, hydroxy, nitro,
trifluoromethyl, C_1 - C_6 straight or branched chain
alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6
alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, and
25 amino.

2. The compound of claim 1, wherein X is CH_2 .

3. The compound of claim 2, wherein:

2-[[[(2-carboxypropyl)hydroxyphosphinyl]methyl]pentane-dioic acid, or a pharmaceutically acceptable salt, hydrate or prodrug thereof.

5 50. A method of inhibiting NAALADase enzyme activity in an animal, comprising administering to said animal an effective amount of a compound of formula I:



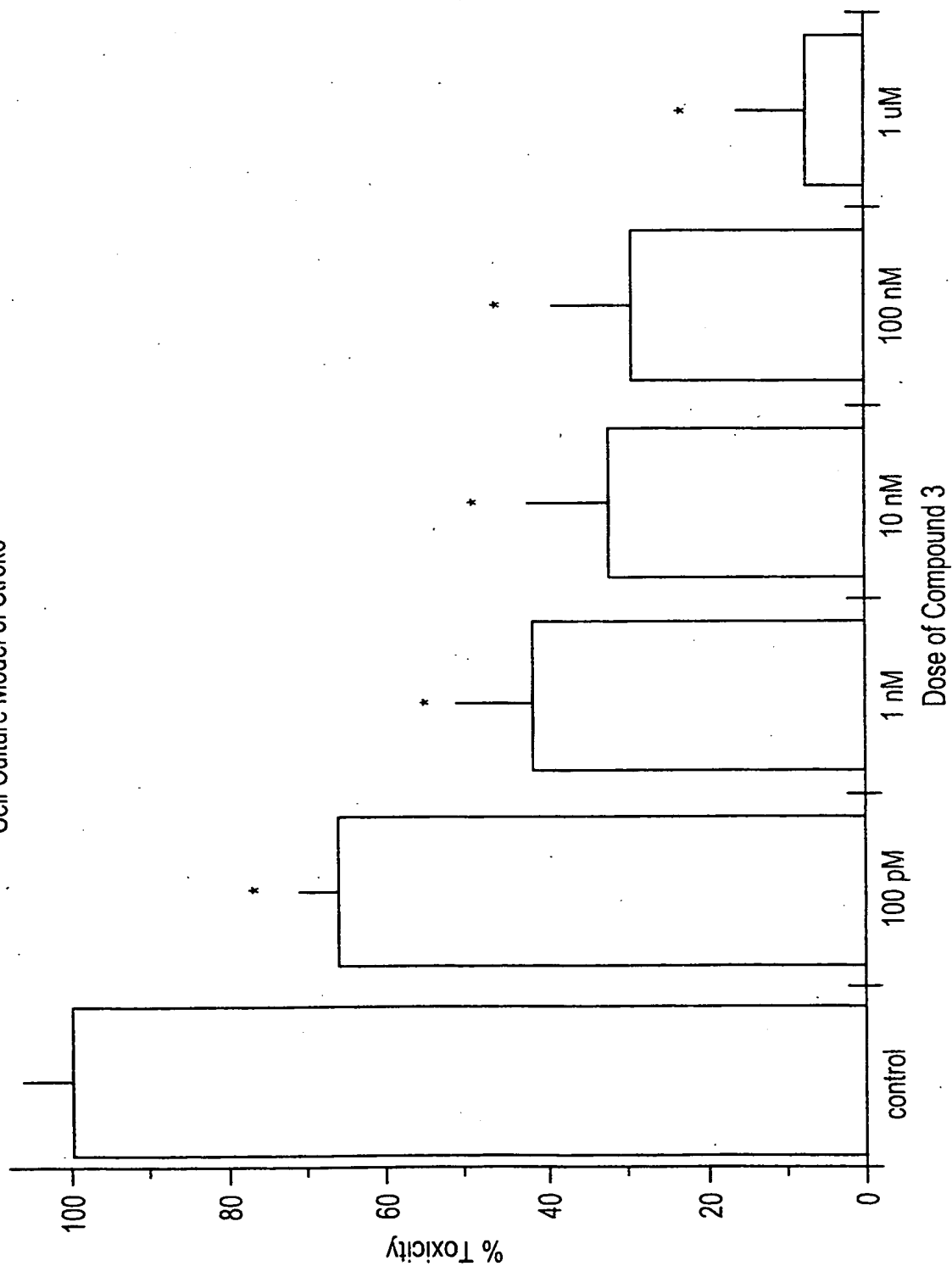
or a pharmaceutically acceptable salt, hydrate or
15 prodrug thereof, wherein:

X is CR₆R₇, O or NR₈;

R₁ is selected from the group consisting of C₁-C₉,
straight or branched chain alkyl, C₂-C₉, straight or
branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇,
20 cycloalkenyl and Ar, wherein said R₁ is unsubstituted
or substituted with one or more substituent(s)
independently selected from the group consisting of
carboxy, carbonyl, C₃-C₈ cycloalkyl, C₅-C₇, cycloalkenyl,
halo, hydroxy, nitro, trifluoromethyl, C₁-C₆, straight
25 or branched chain alkyl, C₂-C₆, straight or branched
chain alkenyl, C₁-C₉, alkoxy, C₂-C₉, alkenyloxy, phenoxy,
benzyloxy, amino, and Ar; provided that when X is -CH₂-
, R₁ is -(CH₂)₂-, R₂ is -(CH₂)₂CO₂H, R₃ is hydrogen, and
R₄ is hydrogen, then R₅ is not hydrogen.

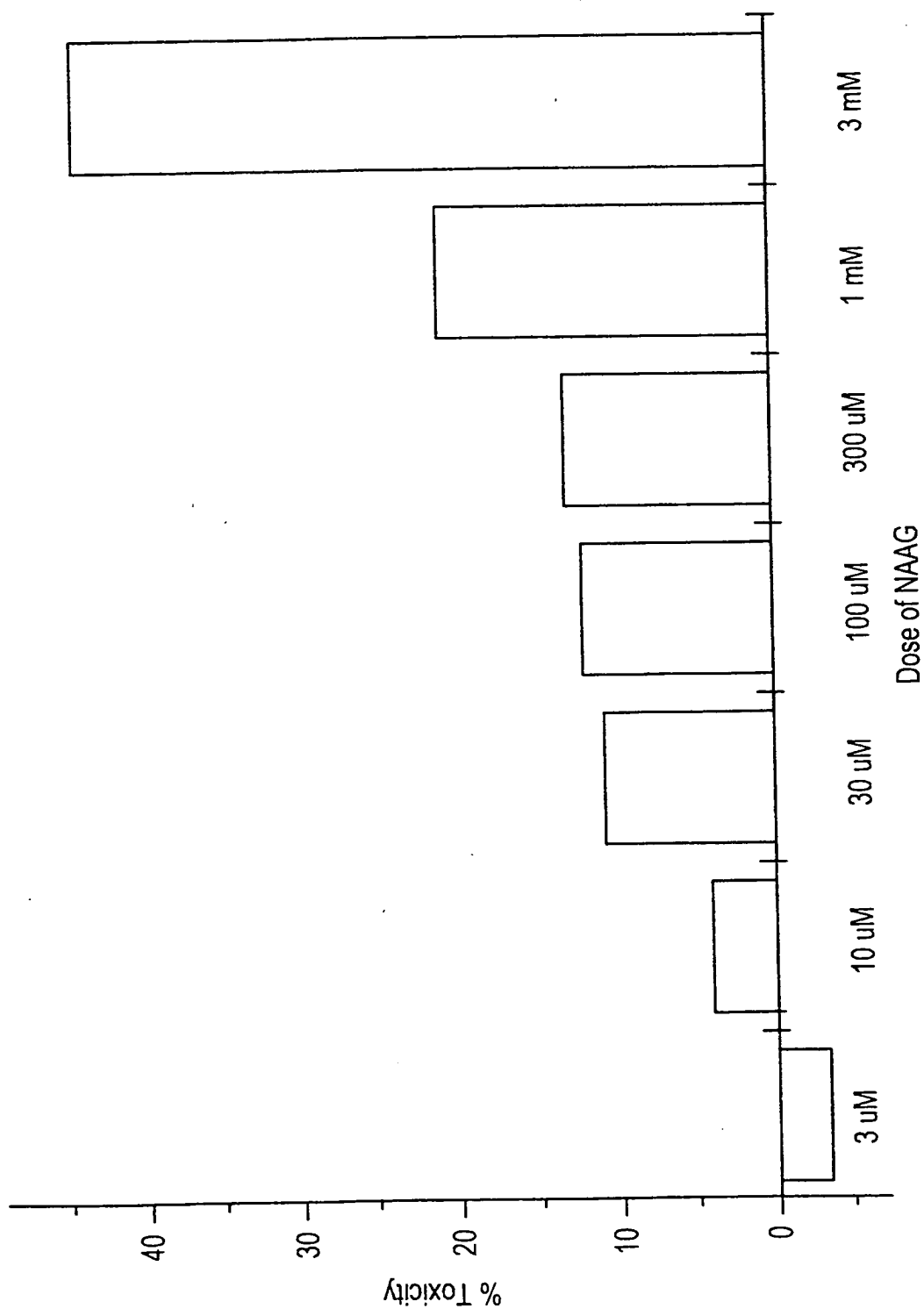
FIG. 1

Compound 3 is Neuroprotective in a
Cell Culture Model of Stroke



SUBSTITUTE SHEET (RULE 26)

FIG. 2
NAAG Toxicity



SUBSTITUTE SHEET (RULE 26)

FIG. 3

NAAG Toxicity Blocked
By Compound 3

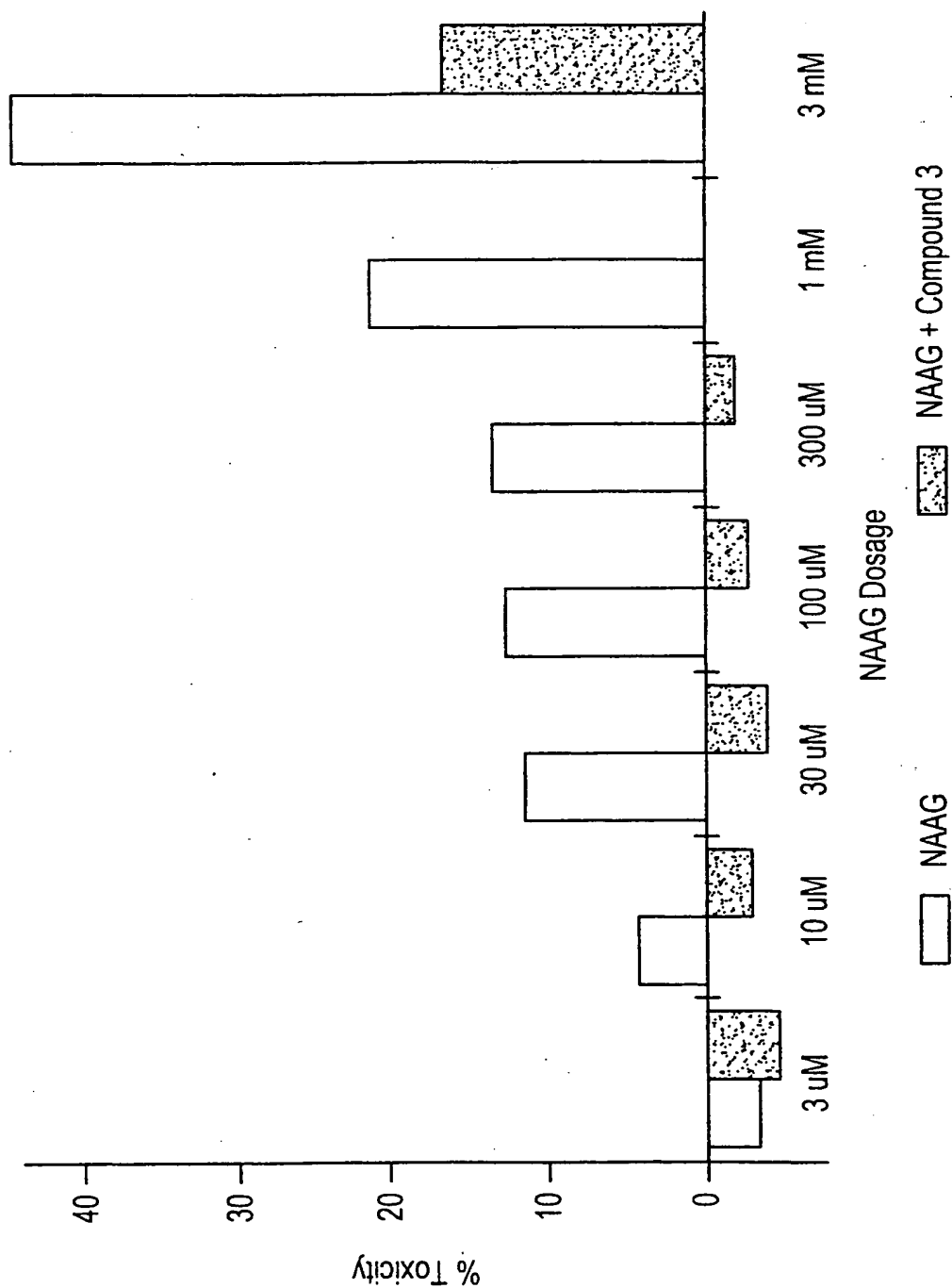


FIG. 4

Compound 3 is Neuroprotective When
Administered Post-Ischemia

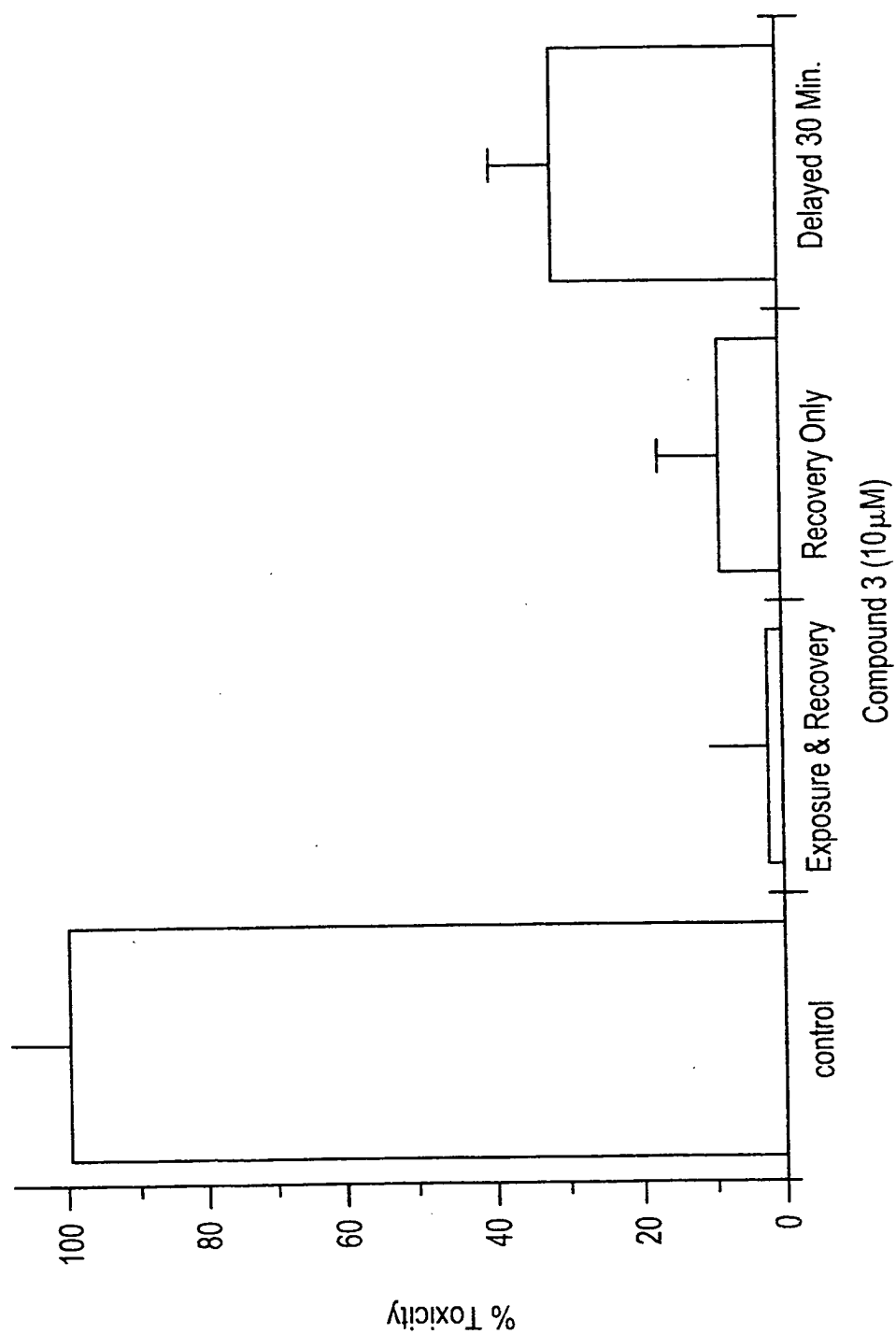
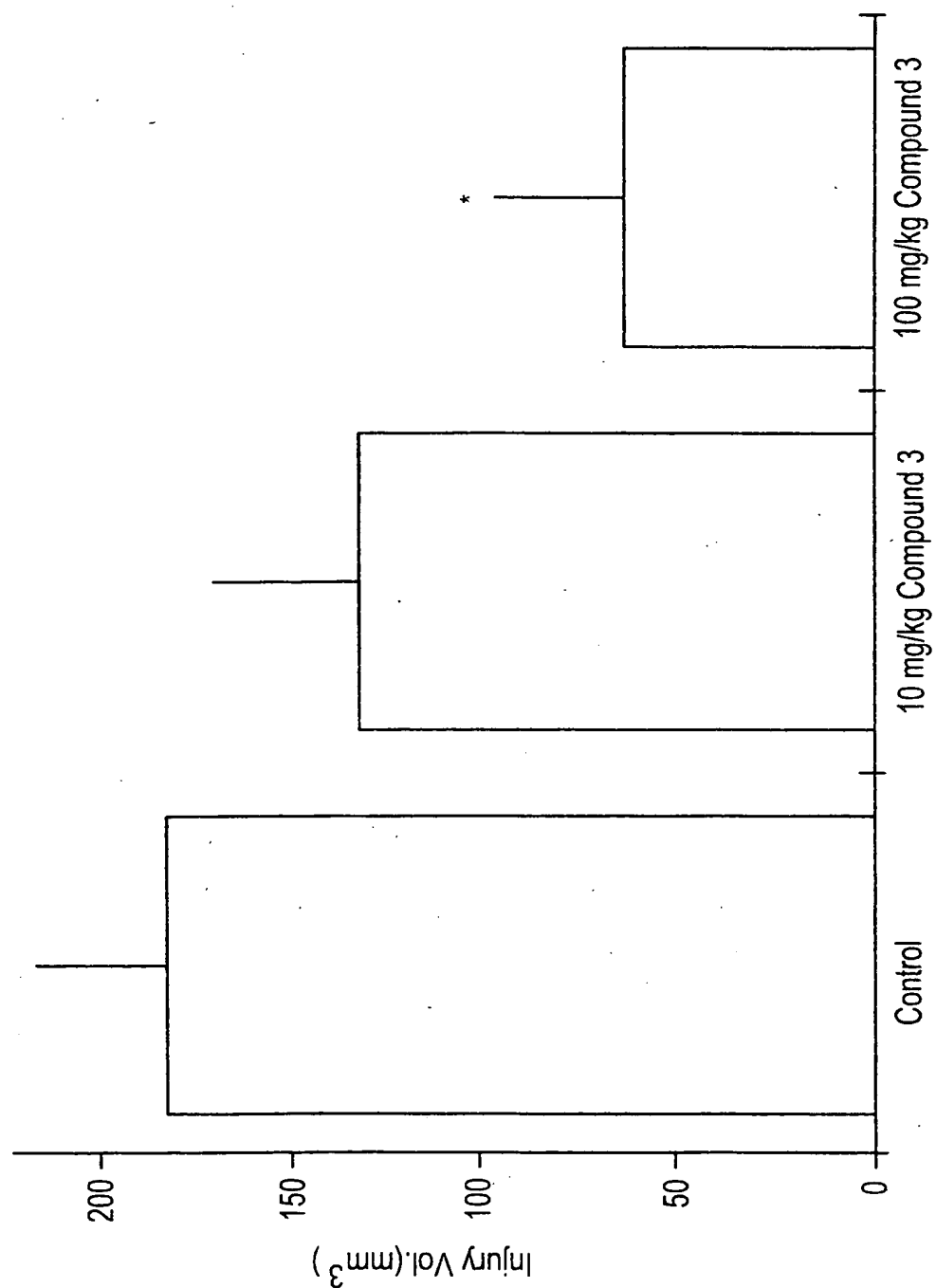


FIG. 5

Compound 3 is Neuroprotective
in Rat MCAO Model of Stroke



SUBSTITUTE SHEET (RULE 26)

FIG. 6

Compound 3 is Neuroprotective in Rat MCAO
Model of Stroke When Administered Up
To 2 Hours Post-Occlusion

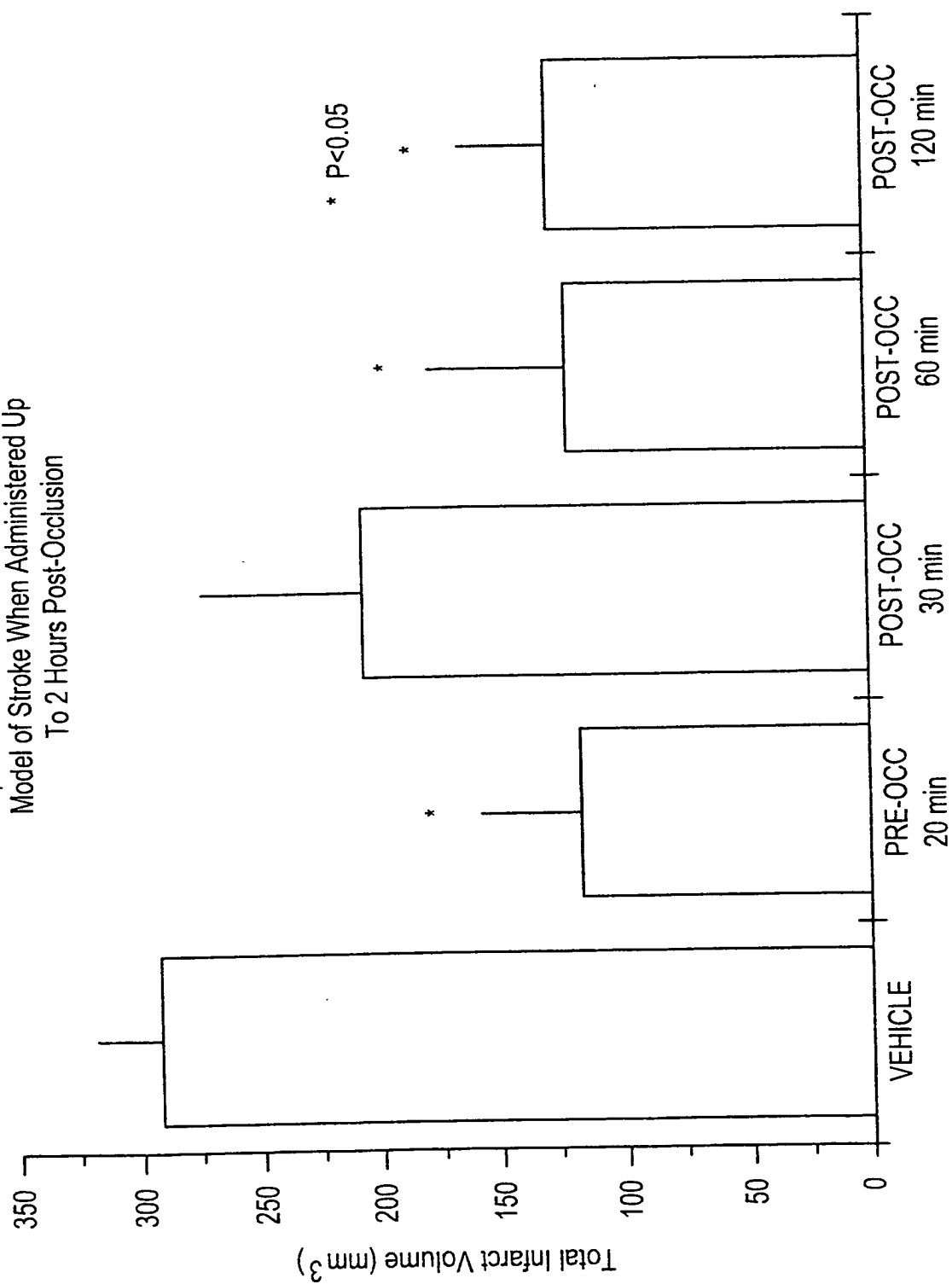


FIG. 7

STRIATUM

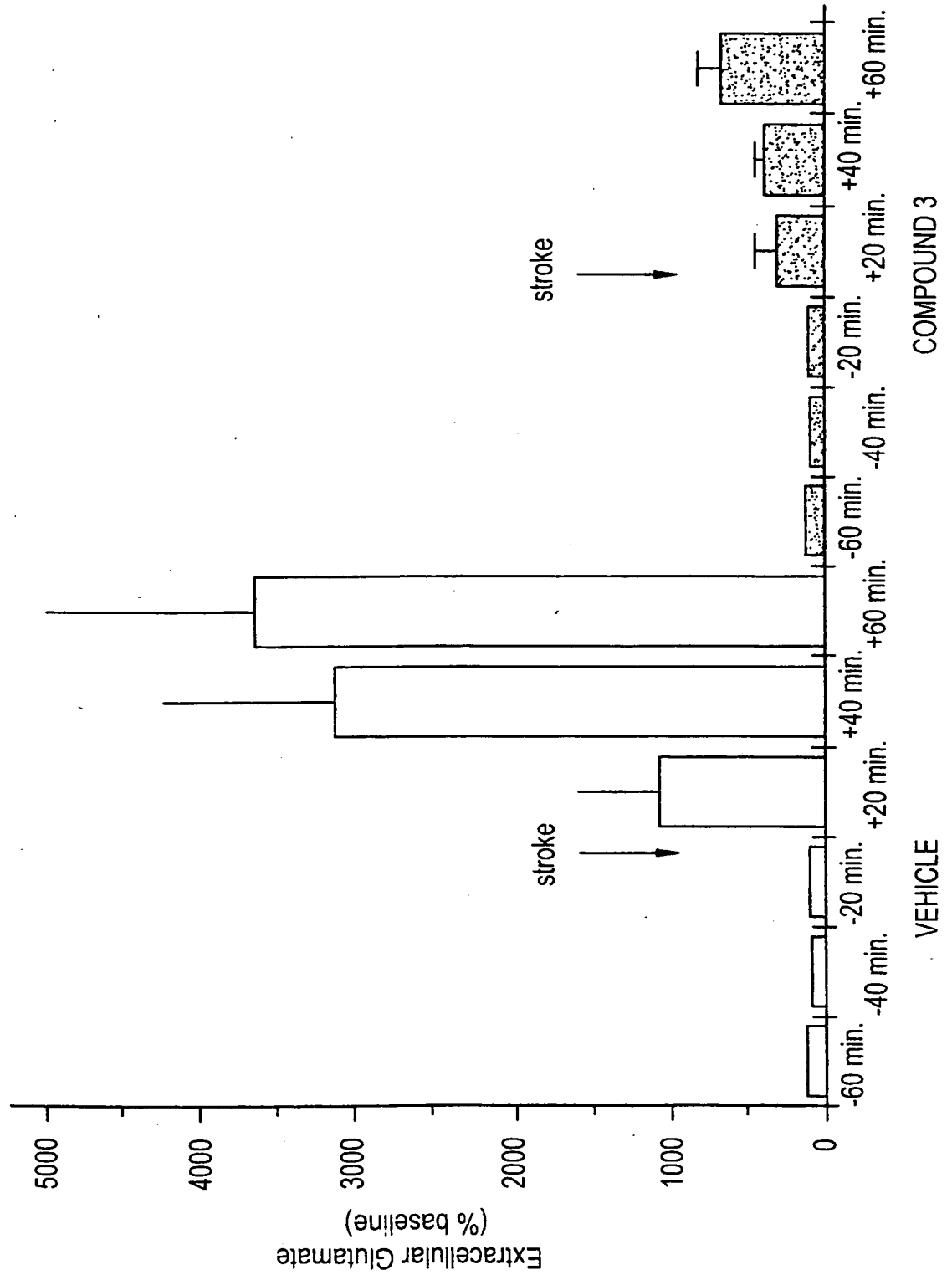


FIG. 8
PARTIETAL CORTEX

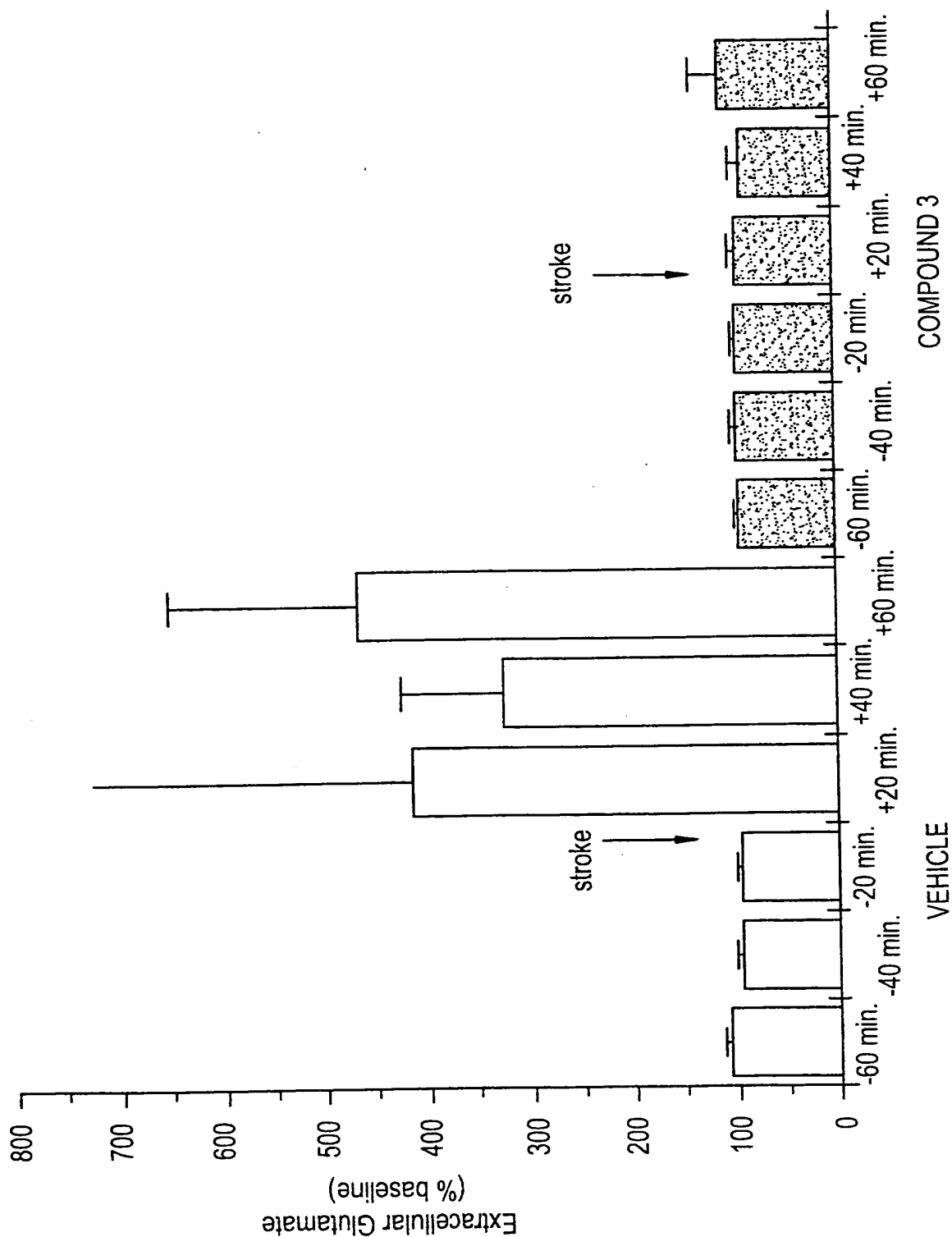
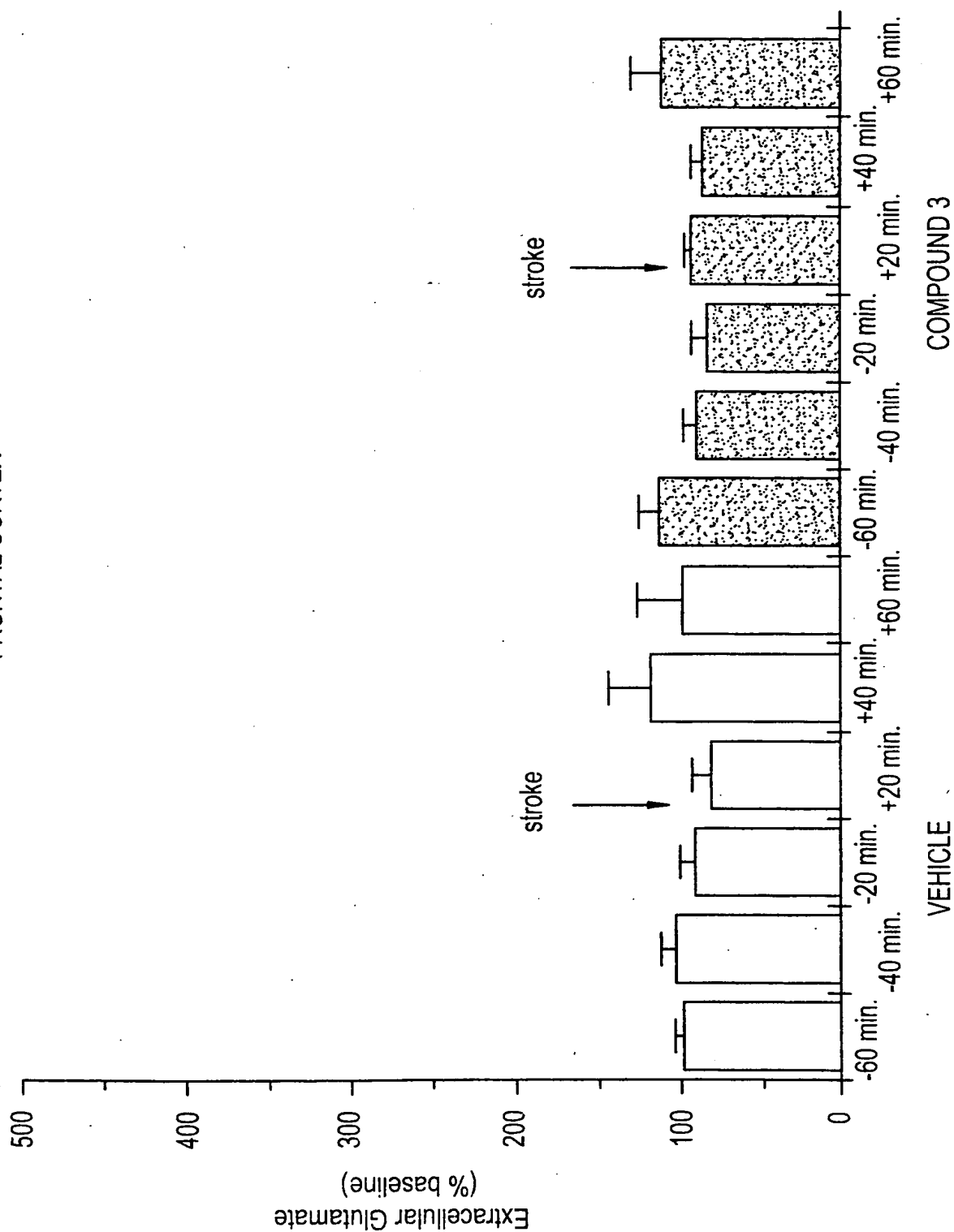
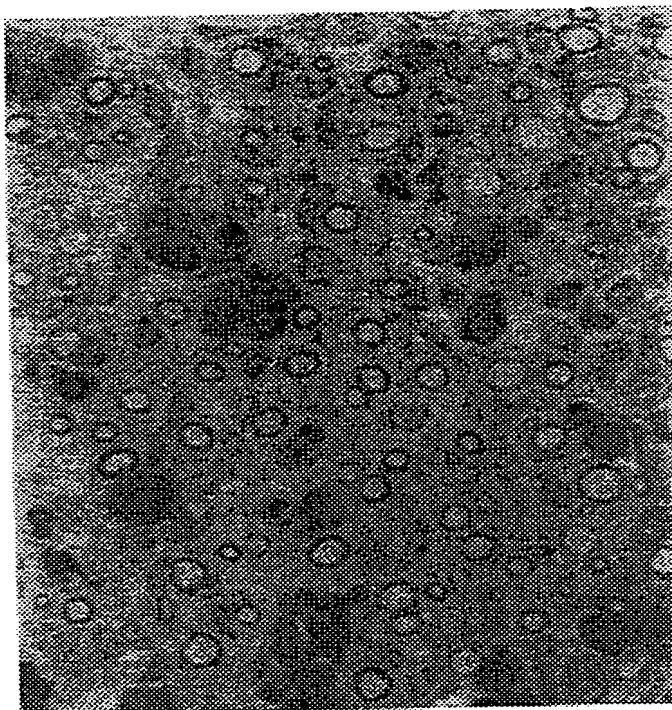


FIG. 9
FRONTAL CORTEX

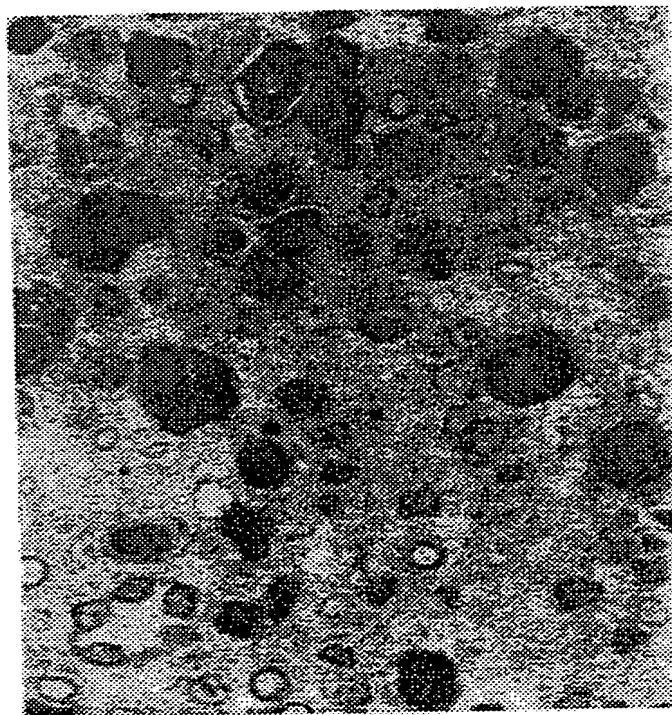


Compound 3 Administration
Following Sciatic Nerve Crush



Compound 3 Polymer
2ug drug/day

FIG.10B



Vehicle Polymer

FIG.10A

FIG. 11

Compound 3 Protects Against MPTP-Toxicity in CD1 Mice

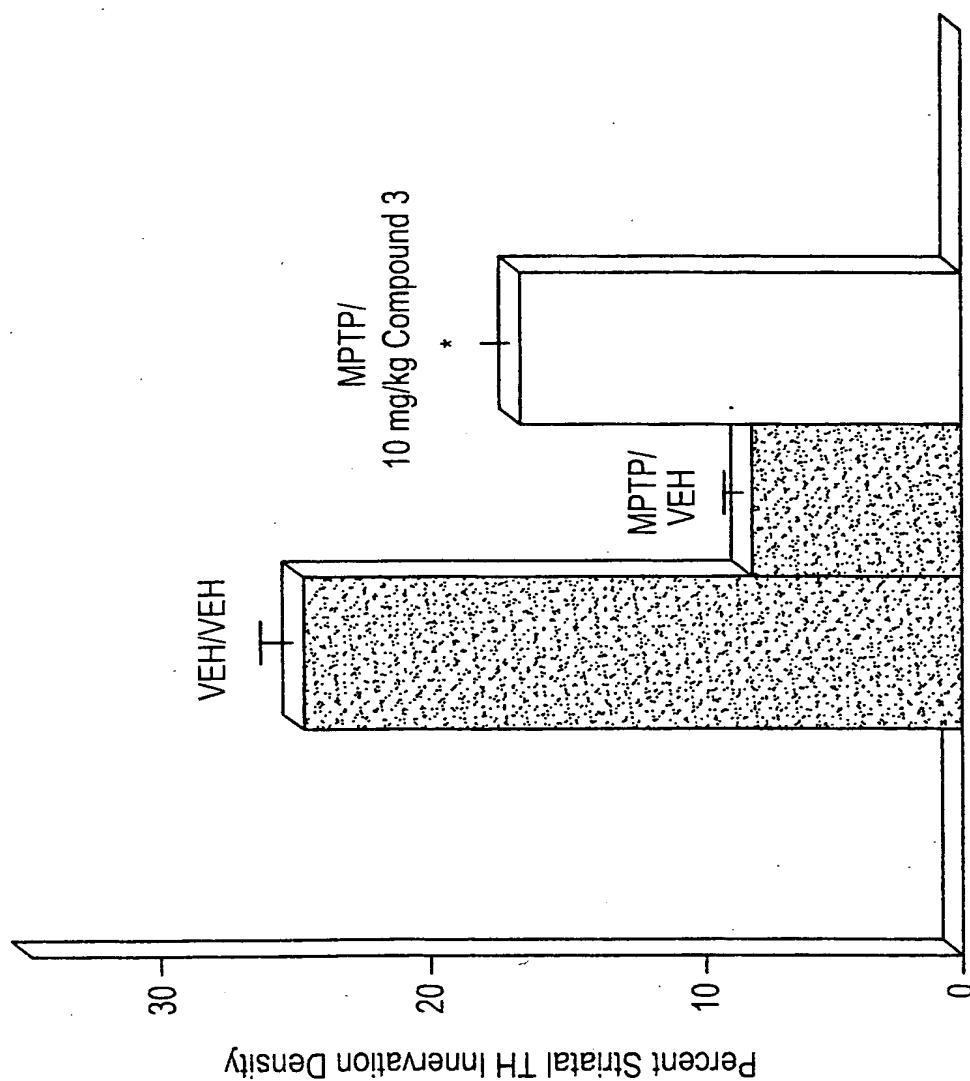


FIG. 12

Compound 3 Is Neuroprotective in a
Dynorphin-Induced Spinal Cord Injury Model

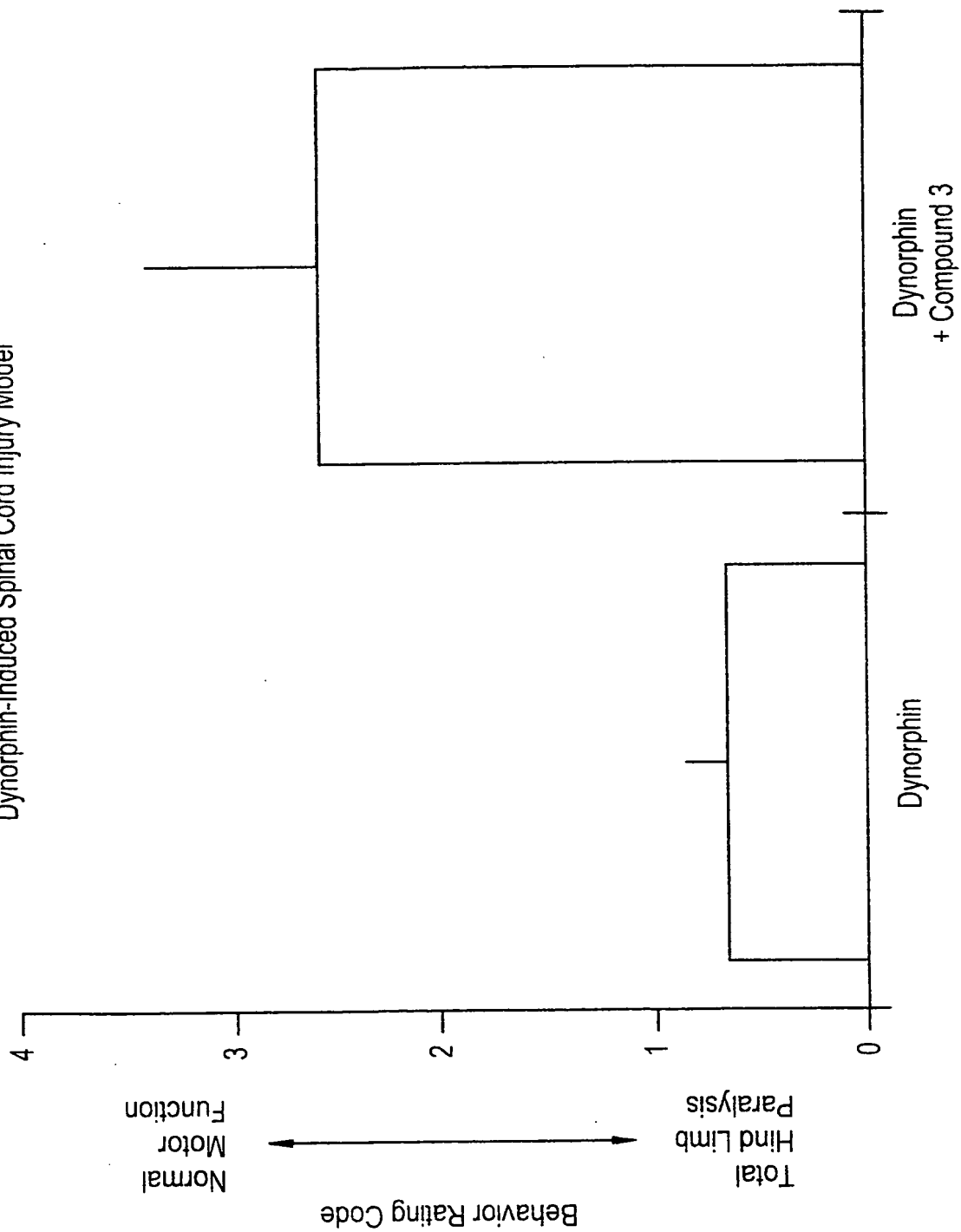


FIG. 13

Compound 3 Is Neuroprotective in a
Spinal Cord Culture Model of ALS

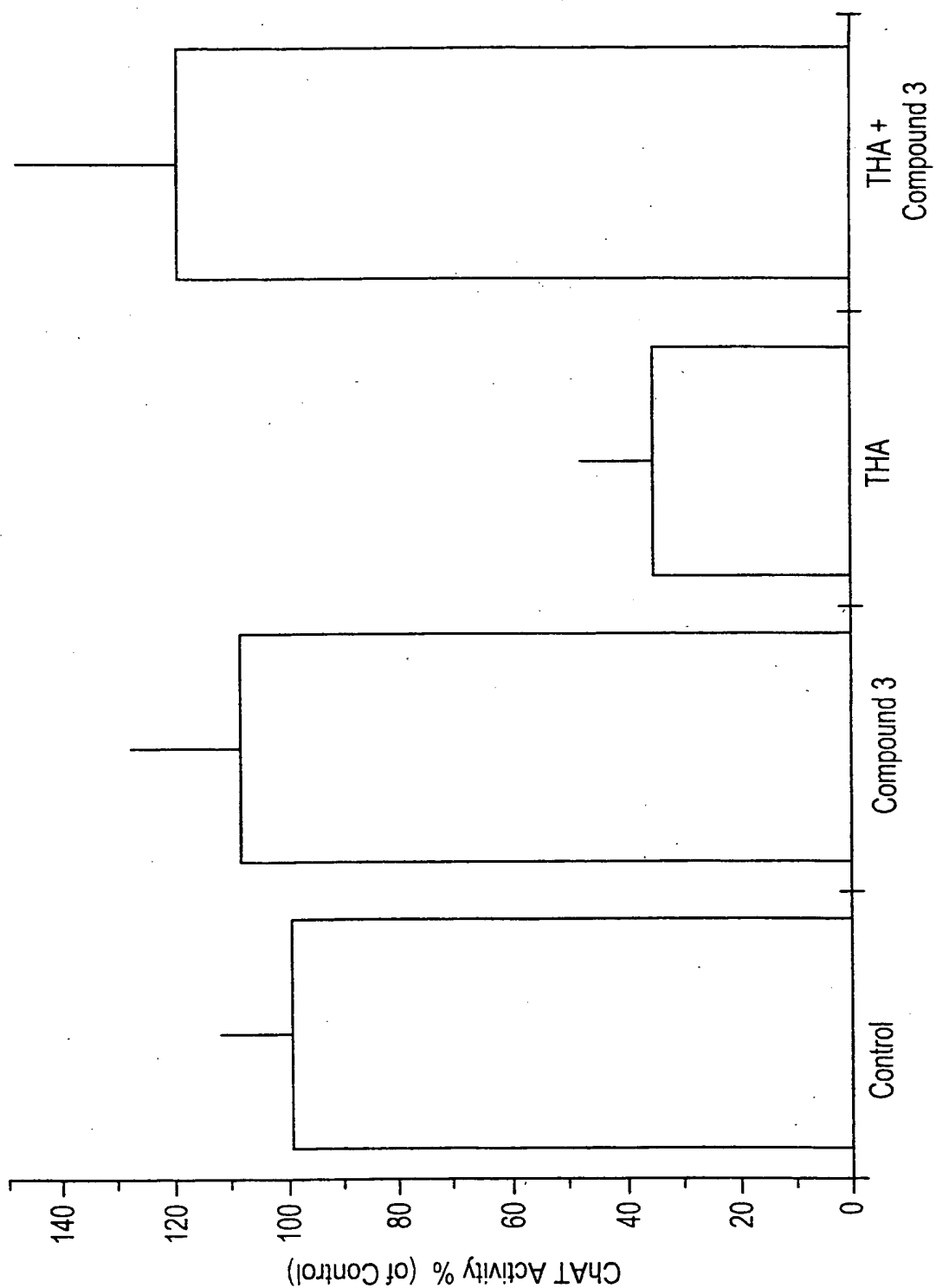


FIG. 14

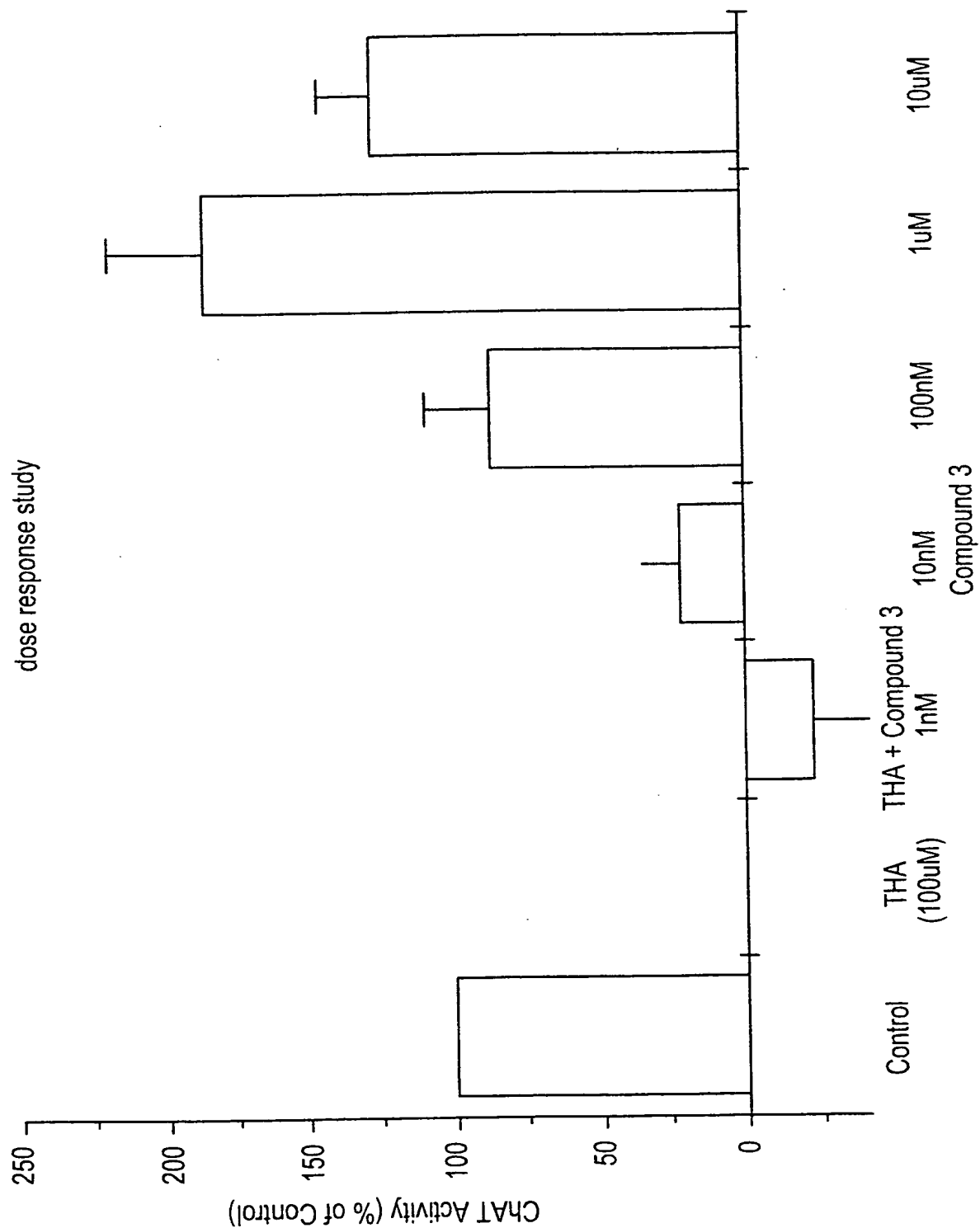


FIG. 15

1 hr ethanol intakes

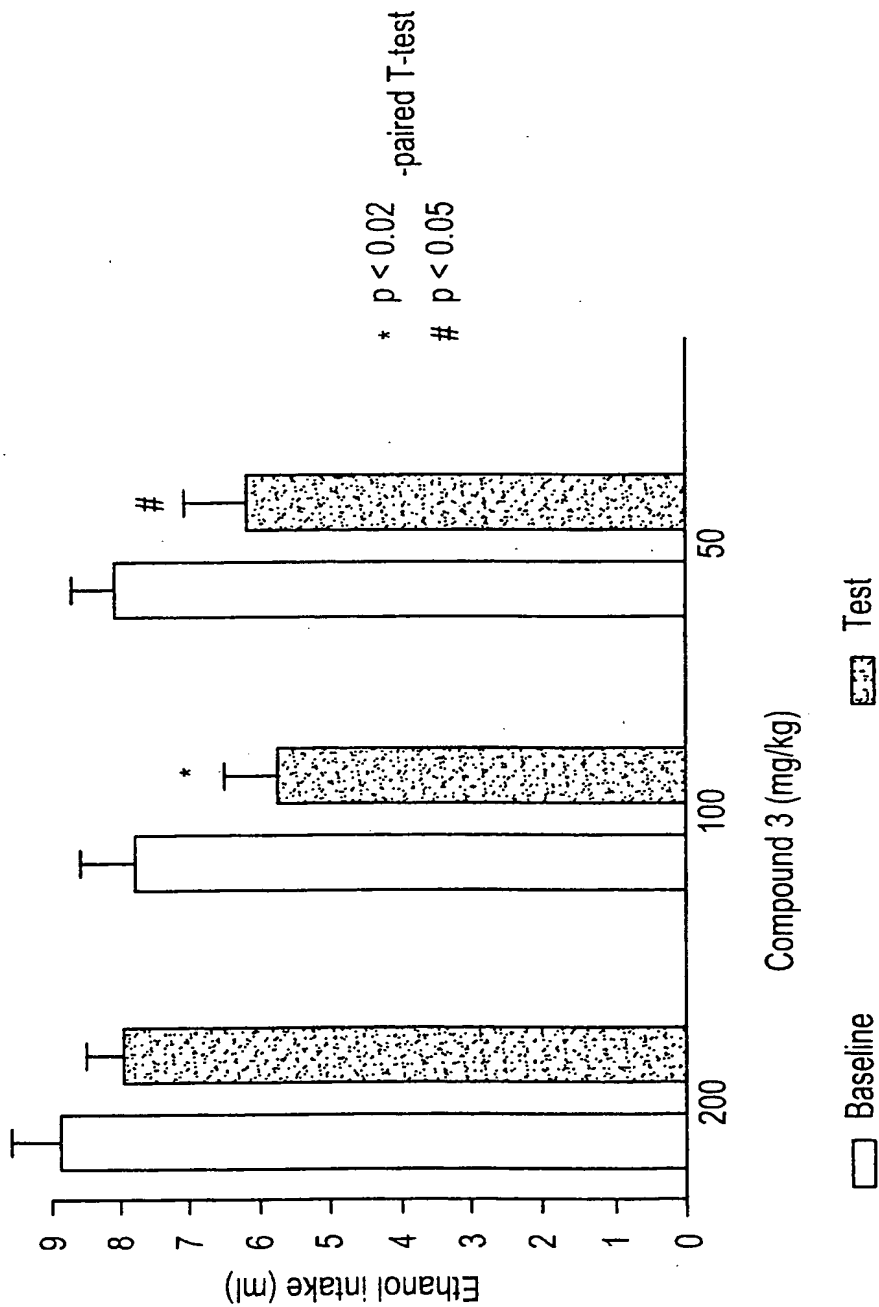


FIG. 16B

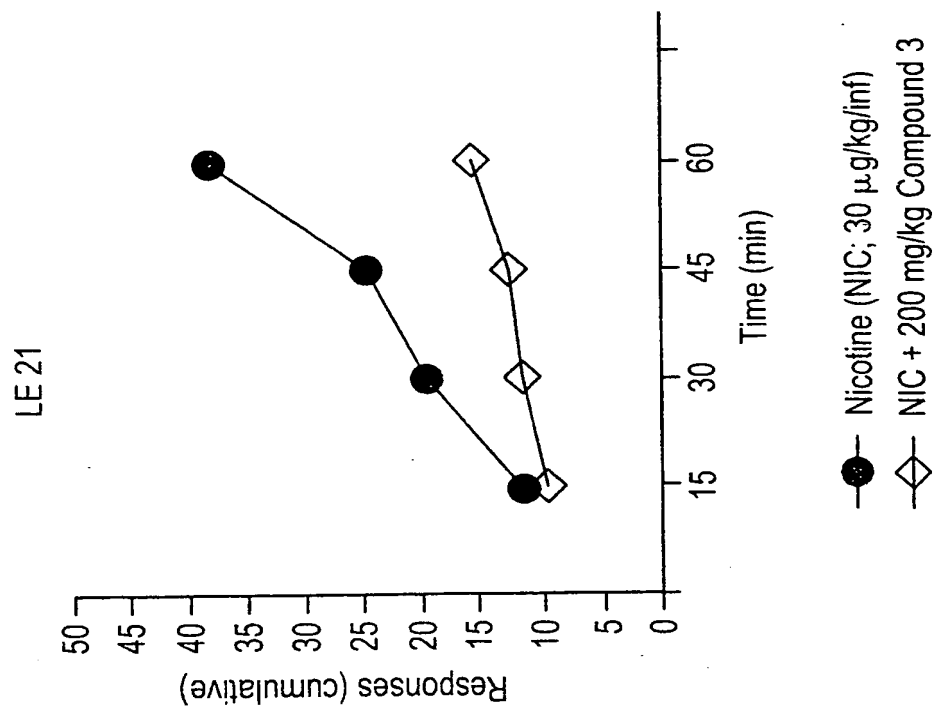
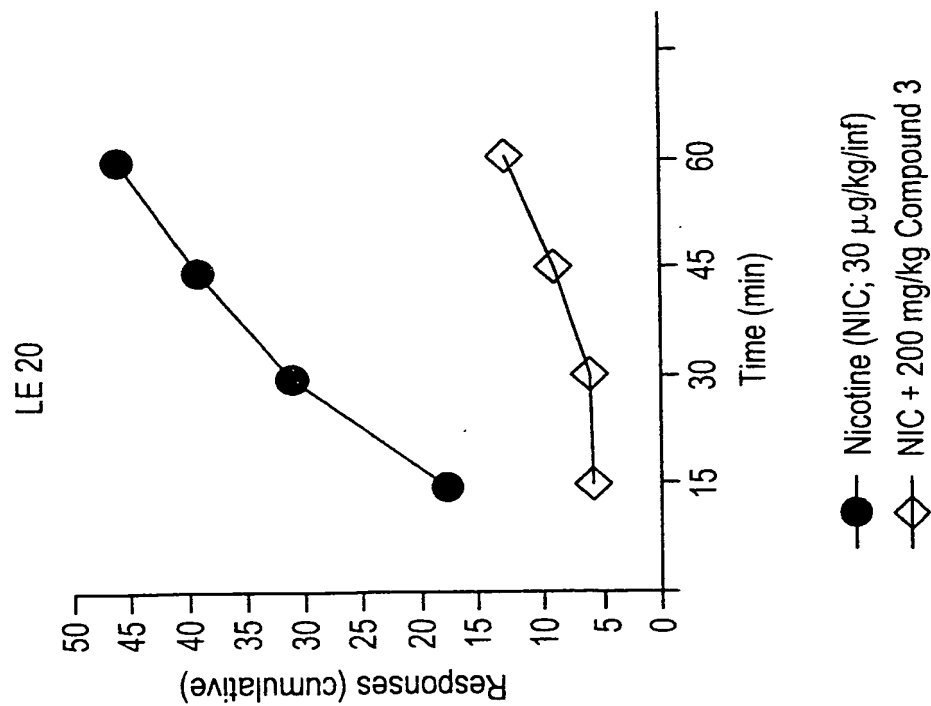


FIG. 16A



SUBSTITUTE SHEET (RULE 26)

FIG. 17B

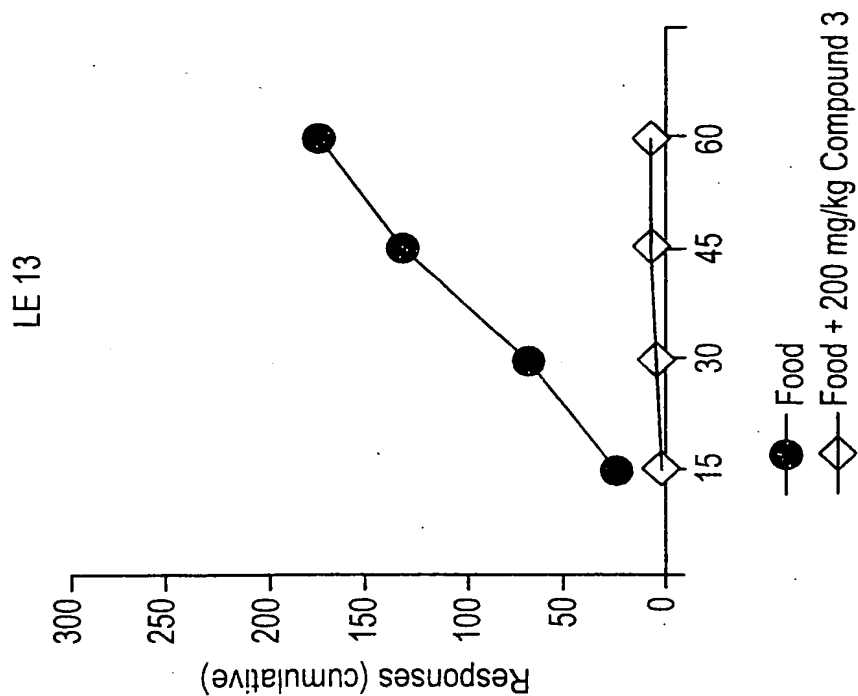


FIG. 17A

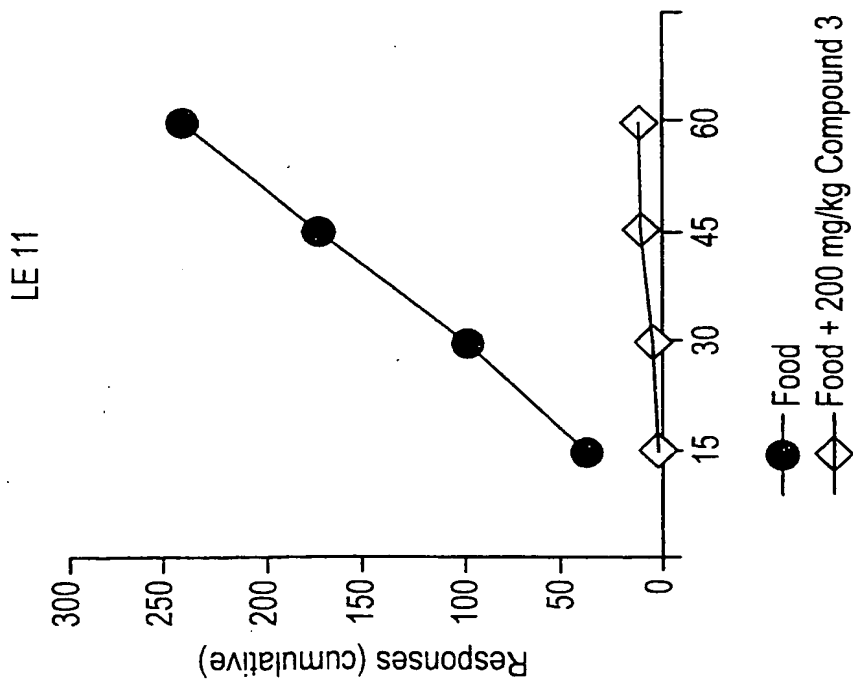


FIG. 18

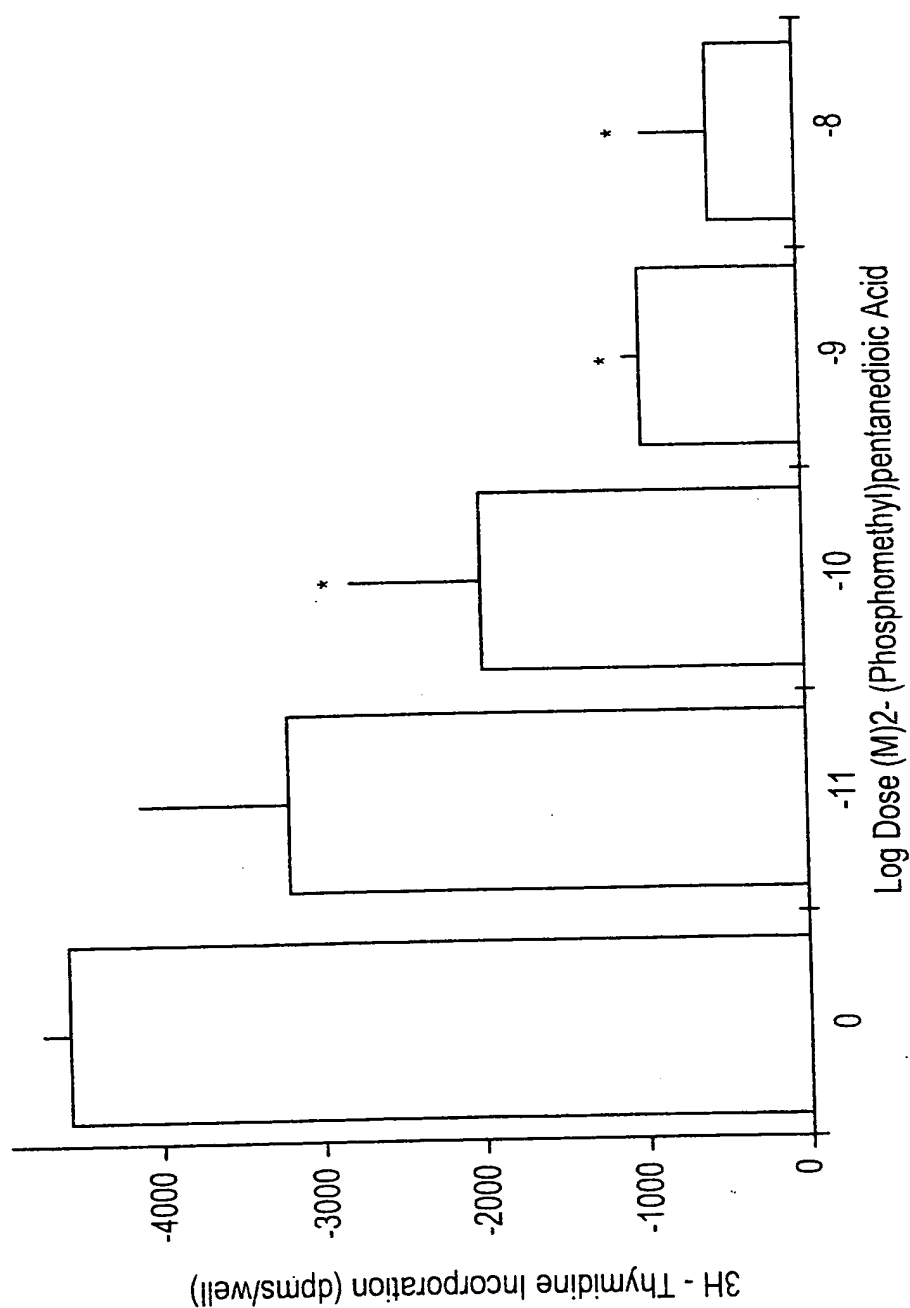
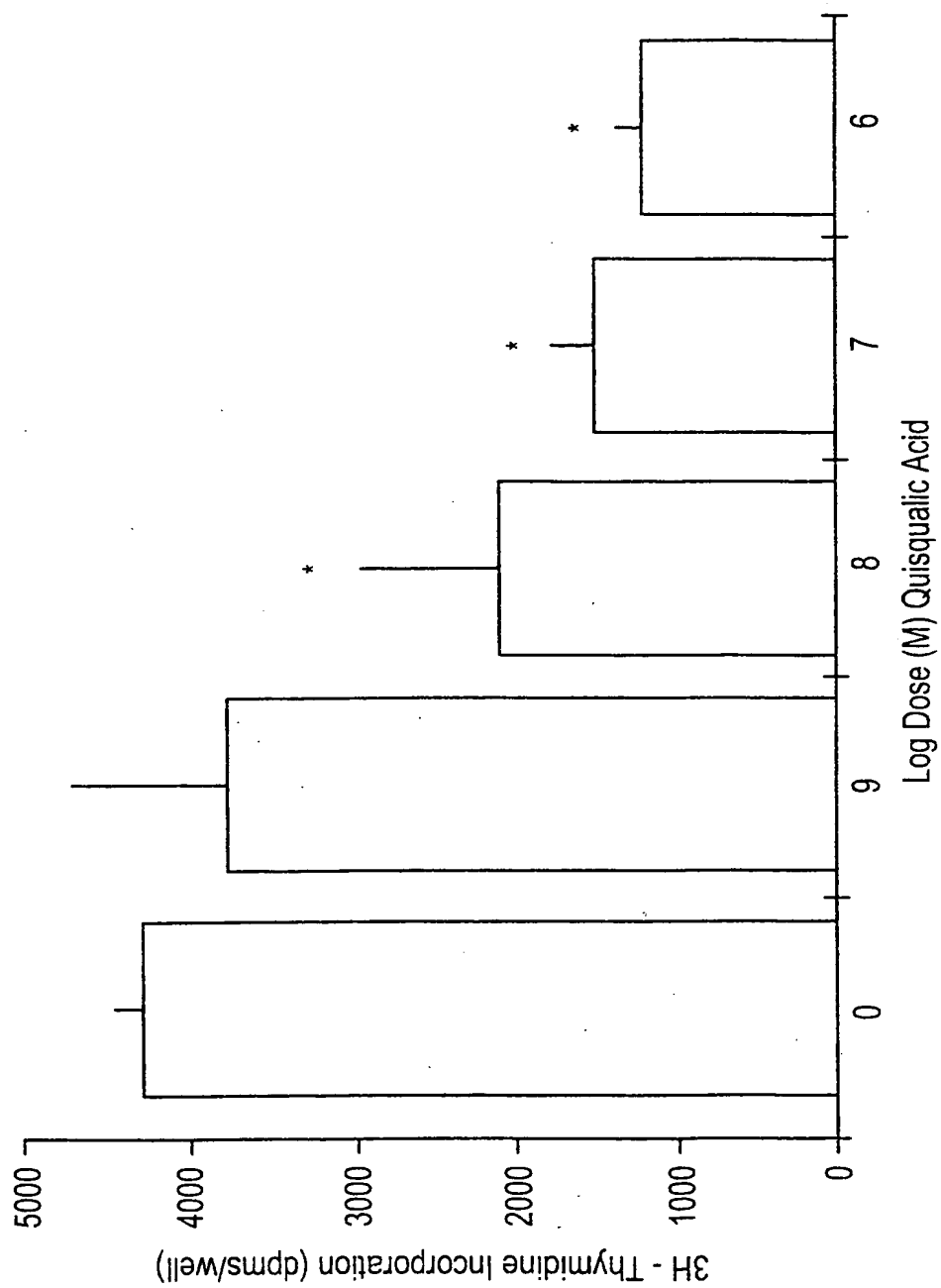


FIG. 19



SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/27175

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07F9/30 A61K31/66 C07F9/58 C07F9/32 C07F9/40
C07F9/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JACKSON P. F.: "Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase N-acetylated alpha-linked acidic dipeptidase" JOURNAL OF MEDICINAL CHEMISTRY., vol. 39, no. 2, - 10 January 1996 pages 619-622, XP002098729 WASHINGTON US see the whole document	1-67
X	WO 97 48400 A (GUILFORD PHARMACEUTICALS INC.) 24 December 1997 see page 72	1-67
P, X	WO 98 13044 A (GUILFORD PHARMACEUTICALS INC.) 2 April 1998 cited in the application see page 21, line 21 and page 84, line 20	1-67
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

1 April 1999

Date of mailing of the international search report

16/04/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Beslier, L

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/27175

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>WO 98 13046 A (GUILFORD PHARMACEUTICALS INC.) 2 April 1998 cited in the application see claims 1,2,5-10,42-49 and 79-85 -----</p>	1-67

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/27175

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9748400 A	24-12-1997	US 5672592 A	30-09-1997
		US 5795877 A	18-08-1998
		US 5863536 A	26-01-1999
		AU 3513597 A	07-01-1998
		AU 3590697 A	07-01-1998
		WO 9748399 A	24-12-1997
		US 5880112 A	09-03-1999
		AU 4151897 A	17-04-1998
		WO 9813044 A	02-04-1998
		AU 3982197 A	30-12-1998
		AU 4067797 A	17-04-1998
		WO 9813046 A	02-04-1998
		WO 9853812 A	03-12-1998
WO 9813044 A	02-04-1998	US 5824662 A	20-10-1998
		US 5795877 A	18-08-1998
		US 5863536 A	26-01-1999
		AU 4067797 A	17-04-1998
		AU 4151897 A	17-04-1998
		WO 9813046 A	02-04-1998
		AU 3513597 A	07-01-1998
		AU 3590697 A	07-01-1998
		WO 9748399 A	24-12-1997
		WO 9748400 A	24-12-1997
		AU 6945198 A	30-10-1998
		WO 9845256 A	15-10-1998
		AU 7100298 A	30-10-1998
		WO 9845257 A	15-10-1998
		AU 6972398 A	13-11-1998
		WO 9847906 A	29-10-1998
WO 9813046 A	02-04-1998	AU 3982197 A	30-12-1998
		WO 9853812 A	03-12-1998
		US 5824662 A	20-10-1998
		AU 4067797 A	17-04-1998
		AU 4151897 A	17-04-1998
		WO 9813044 A	02-04-1998
		AU 6972398 A	13-11-1998
		WO 9847906 A	29-10-1998
		AU 3590697 A	07-01-1998
		AU 3982197 A	30-12-1998
		WO 9748399 A	24-12-1997
		WO 9853812 A	03-12-1998
		AU 3513597 A	07-01-1998
		WO 9748400 A	24-12-1997

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)